Fenotipska obilježja komarče Sparus aurata (Linnaeus, 1758) u prirodnim i antropogeno uvjetovanim okolišima istočnog Jadrana

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PRIRODOSLOVNO-MATEMATIČKI FAKULTET
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Dr.sc. Tanja Šegvić Bubić

Zagreb, 2022.

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FACULTY OF SCIENCE
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PHENOTYPIC TRAITS OF GILTHEAD SEABREAM *SPARUS AURATA* (LINNAEUS, 1758) IN WILD AND HUMAN-MEDIATED ENVIRONMENTS OF THE EASTERN ADRIATIC SEA

DOCTORAL THESIS

Supervisor:
Dr. Tanja Šegvić Bubić

Zagreb, 2022.

Ovaj je doktorski rad izrađen u sklopu Sveučilišnog poslijediplomskog doktorskog studija Geologije pri Geološkom odsjeku, Prirodoslovno-matematičkog fakulteta, Sveučilišta u Zagrebu pod vodstvom dr. sc. Tanje Šegvić Bubić. Istraživanje je provedeno u okviru projekta „Utjecaj akvakulture na prirodne morske populacije (AquaPop)“, podržanog od strane Hrvatske zaklade za znanost (projekt broj IP-2014-09-9050; glavni istraživač dr. sc. Tanja Šegvić Bubić, viša znanstvena suradnica). Prikupljanje biološkog materijala odvijalo se na istočnoj strani Jadranskoga mora, dok su obrada uzoraka, analiza podataka i pisanje doktorskog rada izvedeni na Institutu za oceanografiju i ribarstvo u Splitu, u Republici Hrvatskoj.

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Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Geološki odsjek

Doktorski rad

FENOTIPSKA OBILJEŽJA KOMARČE *SPARUS AURATA* (LINNAEUS, 1758) U PRIRODNIM I ANTROPOGENO UVJETOVANIM OKOLIŠIMA ISTOČNOG JADRANA

IGOR TALIJANČIĆ

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Ribe, kao živi organizmi, izrazito su prilagodljive prirodnim i antropogeno uvjetovanim okolišima gdje svojstvo fenotipske plastičnosti omogućava poveznicu starih i novih sredina putem morfoloških, fizioloških prilagodbi ponašanja. Posljednjih godina zamijećeno je povećanje ulova divlje komarče na području istočnog Jadranskog mora, gdje riblji zbjegovi i priljev jedinki iz kavezno povezanih okoliša uzgajališta tuna doprinose povećanju brojnosti populacija u prirodnom staništu. Predmetnim istraživanjem vrednovane su fenotipske prilagodbe kod komarče u okolišu pod utjecajem kaveznog uzgoja tuna te fenotipska obilježja divljih i uzgojnih populacija s ciljem razvoja brzog i učinkovitog alata za raspoznavanje zbjegova u lovini. Primjenom metoda geometrijske morfometrije kvantificirana su morfološka obilježja tijela i otolita, dok je fiziološki odgovor promatran kroz trofički status i spolna obilježja protandričnih populacija. Cjelogodišnja prostorna i vremenska zastupljenost komarči oko uzgajališta tuna upućuje na važnu ulogu jadranskih uzgajališta tuna kao pogodnih staništa za divlju komarču. Taj izmijenjeni okoliš u kojem prevladava drugačija dinamika morskih struja i dostupnost plijena uvjetovao je specifičan odgovor kod komarče formirajući novi kavezno povezani morfotip. Manja i tuplja glava te vretenastije tijelo predstavljaju osnovna obilježja spomenutog morfotipa. U blizini kaveza tuna dokumentirana je promjena spola komarče pri nižim dužinskim veličinama u odnosu na prirodne populacije zbog značajne trofičke podrške koja osigurava bolji kondicijski i reproduktivni status. Promatrajući divlje i uzgojne komarče, primjenom metoda geometrijske morfometrije uspješno su klasificirane jedinke prema izvornom podrijetlu u iznosu od 99,6 % s obzirom na oblik tijela te više od 70 % u odnosu na oblik otolita. Zaokruženiji profil glave sa supraterminalnim pozicioniranim ustima te zdepast oblik tijela s tamnijom i bljeđom srebrnkasto-sivom obojenošću glavna su obilježja uzgojnih komarči, dok su divlje jedinke opisane s izduženijim tijelom i subterminalnim ustima na većem profilu glave. Prikupljene informacije primjenjive su u budućim biotehnološkim istraživanjima za potrebe prepoznavanja podrijetla ribe strojnim učenjem i dugoročno u upravljanju zbjegovima te u razvoju selekcijskih programa vrsta iz akvakulture.

(146 stranica, 12 tablica, 8 slika, 207 literaturna navoda, jezik izvornika: hrvatski)

Ključne riječi: akvakultura, komarča, fenotip, plastičnost, riblji zbjegovi, uzgajališta tuna

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Fish, as living organisms, are highly adaptable to natural and human-mediated environments due to their morphological, physiological and behavioural adaptations through phenotypic plasticity. In recent years, a increase of gilthead seabream in fisheries landings has been documented in the eastern Adriatic Sea, with aquaculture escapes and spillover of seabream from tuna farm concession areas contributing to population increases in natural habitats. The aim of this research was to investigate the phenotypic adaptations of gilthead seabream in a tuna farm impacted environment and to evaluate the phenotypic characteristics of wild and farmed populations in order to develop a rapid and effective tool for detecting escapes in fisheries landings. Morphological characteristics of body and otolith shape were quantified by geometric morphometrics methods, while physiological responses were studied through trophic status and sexual characteristics of protandrous populations. The year-round spatial and temporal occurrence of seabream near tuna farms indicates that they play an important role as suitable habitat for wild seabream populations in the Adriatic Sea. This altered environment, characterised by different hydrodynamics and prey availability compared to natural habitats, has triggered a specific phenotypic response of seabream by forming a new morphotype associated with tuna farms. The smaller and blunt head and the more spindle-shaped body are the main characteristics of the mentioned morphotype. In the vicinity of tuna farms, the sex change of seabream has been documented at lower lengths compared to wild populations, due to the significant trophic support that ensures better condition and reproductive status. When observing wild and farmed seabream, geometric morphometrics methods successfully assigned 99.6% of individuals to their correct origin in terms of body shape and more than 70% in terms of otolith shape. A rounder head profile with a supraterminal positioned mouth and a stocky body shape with darker and paler silver-grey coloration are the main characteristics of farmed seabream, while wild individuals are described with an elongated body and a subterminal mouth on a larger head profile. The information collected in this research can be used in future biotechnological research to identify the origin of fish through machine learning and, in the long term, in the management of escapes and the development of selection programmes for aquaculture species.

(146 pages, 12 tables, 8 figures, 207 references, original in Croatian)

Keywords: aquaculture, gilthead seabream, phenotype, plasticity, escapes, tuna farms

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SADRŽAJ

POPIS ZNANSTVENIH RADOVA

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II. **Talijančić, I.**, Šegvić-Bubić, T., Žužul, I., Džoić, T., Maršić-Lučić, J., Grubišić, L. (2019) Morphological and ecophysiological adaptations of wild gilthead seabream *Sparus aurata* associated with tuna farms. *Aquaculture Environment Interactions*, 11: 97-110. doi: 10.3354/aei00294

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UVOD

Porast potražnje za proizvodima ribarstva izražen je već desetljećima zbog povećanja brojnosti stanovništva te osvješćivanja potrošača o prehrambenim navikama koji vode zdravijem načinu življenja. Ribe i ribama slične namirnice predstavljaju važan izvor prehrane gdje prosječan unos bjelančevina ribljeg podrijetla iznosi oko 17 % od unošenih bjelančevina životinjskog podrijetla te 7 % od ukupno svih konzumiranih (FAO[1], 2020). Osim u bjelančevinama, zdravstvene dobrobiti očituju se i u sadržaju nezasićenih omega-3 masnih kiselina, vitaminima i mineralima poput željeza, cinka odnosno svih onih biokemijskih sastavnica koje su neophodne za postizanje što boljeg ljudskog zdravlja (Beveridge i sur., 2013; Wheal i sur., 2016).

Svjetska opskrba ribarstvenim proizvodima više se nego udvostručila u razdoblju od 1980-ih do 2018. godine, kada je zabilježen porast sa 72 na 156 milijuna t, i prosječne godišnje potrošnje ribe s 13,4 kg na trenutnih 20,5 kg po glavi stanovnika (FAO, 2020). Prema predviđanjima budućeg rasta potrošnje, do 2030. godine očekuje se daljnje povećanje proizvodnih kapaciteta na 204 milijuna tona, uz nastavak trenda stabilizacije ribolova na oko 90 milijuna t godišnje te porast uzgoja vodenih organizama na 109 milijuna t. Uznapredovale ribolovne tehnologije i tehnike te povećani ribolovni napor ne mogu više zadovoljiti sve veće potrebe za proizvodima ribarstva, s obzirom na to da je trenutno 59 % morskih ribljih stokova u statusu iskorištavanja do razine maksimalnog održivog ulova, dok je 34 % u potpunosti iskorišteno (FAO, 2020). Stoga se razvoj uzgoja vodenih organizama nameće kao buduće rješenje jer osigurava cjelogodišnju dostupnost proizvoda te smanjuje ribolovni pritisak na prelovom ugroženim prirodnim ribljim naseljima, čime se doprinosi njihovoj održivosti i zaštiti biološke raznolikosti (Katavić, 2017).

Iako svjetska akvakulturna proizvodnja ne bilježi veću stopu godišnjeg rasta od one 1980-ih i 1990-ih (10,8 % i 9,5 %), i dalje je najbrže rastući sektor proizvodnje hrane u svijetu, s godišnjom prosječnom stopom rasta od 5,8 % u periodu od 2001. do 2010. i 4,5 % u razdoblju 2011.–2018. godine. U posljednjem su razdoblju prepoznate okolnosti koje su utjecale na smanjenu stopu rasta svjetskog uzgoja: (i) uspostava strožih okolišnih uredbi prilikom rukovođenja uzgojnih aktivnosti; (ii) smanjena dostupnost uzgojnih slatkovodnih lokacija i drugih proizvodnih područja te (iii) učestalija pojava bolesti u intenzivnim uzgojnim jedinicama (FAO, 2020). Ukupni povijesni rekord proizvodnje od 114,5 milijuna t ostvaren je u 2018.

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[1] FAO (eng. *Food and Agriculture Organization*) – Organizacija za hranu i poljoprivredu Ujedinjenih naroda.

godini, od čega je 82,1 milijuna t akvatičnih životinja i 32,4 milijuna t algi namijenjeno ljudskoj prehrani (Tablica 1). Na globalnoj razini sastav uzgojnih vrsta razlikuje se u ovisnosti o slatkovodnom ili morskom uzgojnom mediju. Ukupna proizvodnja slatkovodnih vrsta iznosila je 51,3 milijuna t. Ribe i rakovi bili su dominantno zastupljene uzgojne vrste s udjelima od 91,5 % te 7,1 %, dok je uzgoj svih drugih organizama iznosio 1,4 %. Ukupna proizvodnja morskih organizama iznosila je 30,1 milijuna t, od čega su mekušci i morske ribe bile glavne uzgojne vrste sa zastupljenošću od 56,2 % te 23,8 %, dok je uzgoj drugih morskih organizama iznosio 20 %.

Tablica 1. Svjetska proizvodnja akvatičnih organizama u akvakulturi i ribarstvu, bez vodenog bilja (Izvor: FAO, 2020).

A) PROIZVODNJA	1986. – 1995.	1996. – 2005.	2006. – 2015.	2016.	2017.	2018.
	(u milijunima tona)					
Ulov						
Unutarnje vode	6,4	8,3	10,6	11,4	11,9	12,0
More	80,5	83,0	79,3	78,3	81,2	84,4
Sveukupno	86,9	91,4	89,8	89,6	93,1	96,4
Akvakultura						
Unutarnje vode	8,6	19,8	36,8	48,0	49,6	51,3
More	6,3	14,4	22,8	28,5	30,0	30,8
Sveukupno	14,9	34,2	59,7	76,5	79,5	82,1
Ukupno svjetsko ribarstvo	101,8	125,6	149,5	166,1	172,7	178,5
B) ISKORIŠTAVANJE						
Ljudska prehrana	71,8	98,5	129,2	148,2	152,9	156,4
Ostala upotreba	29,9	27,1	20,3	17,9	19,7	22,2
Populacija (u milijardama)	5,4	6,2	7,0	7,5	7,5	7,6
Po glavi stanovnika (kg)	13,4	15,9	18,4	19,9	20,3	20,5

Akvakultura Europske unije (EU) stagnira te je i dalje daleko od ostvarenja punog potencijala u smislu rasta i zadovoljavanja sve veće potrebe za održivijim morskim prehrambenim proizvodima. Naime, EU uvozi više od 70 % svoje potrošnje morskih prehrambenih proizvoda budući da vlastita proizvodnja namijenjena ljudskoj prehrani od 5,3 milijuna t pokriva samo 42 % potražnje, od čega ribolov sudjeluje s 32 %, a akvakultura s 10,6 % udjela. Prosječna potrošnja proizvoda ribarstva i akvakulture po glavi stanovnika u 2018. godini iznosila je 24,36 kg, od čega su proizvodi divljeg podrijetla sudjelovali sa 74,3 %

ukupnog iznosa (EUMOFA[2], 2020). Sagledavajući protekli desetogodišnji period, proizvodnja u akvakulturi narasla je za 3 % te je u 2018. godini iznosila 1,32 milijuna t.

Proizvodnja EU-a uključuje uzgoj (i) morskih vrsta riba s najznačajnijim vrstama, a to su atlantski losos (*Salmo salar*, Linnaeus 1758), tuna (*Thunnus thynnus*, Linnaeus 1758), lubin (*Dicentrarchus labrax*, Linnaeus 1758) i komarča (*Sparus aurata*, Linnaeus 1758), (ii) školjkaša gdje prevladavaju vrste mediteranska dagnja (*Mytilus galloprovincialis*, Lamark 1819) i pacifička kamenica (*Magallana gigas*, Thunberg 1793) te (iii) slatkovodnih vrsta riba, ponajviše kalifornijske pastrve (*Oncorhynchus mykiss*, Walbaum 1792), šarana (*Cyprinus carpio*, Linnaeus 1758) i jegulje (*Anguilla anguilla*, Linnaeus 1758). Od ukupne 1,32 milijuna t uzgojne biomase najveći dio zauzima uzgoj dagnji (36 %), zatim kalifornijske pastrve (14 %), lososa (13 %), komarče i lubina (14 %) te kamenice (8 %).

Na tržištu EU-a najzastupljeniji proizvodi morske akvakulture iz mediteranskih su zemalja poput Španjolske, Francuske, Italije i Grčke. U skladu s globalnim trendovima, proizvodnja u Sredozemlju napredovala je 90-ih godina prošlog stoljeća te danas ima važnu društvenu i gospodarsku ulogu u regiji. Zadovoljava povećanu potražnju za morskim prehrambenim proizvodima te znatno doprinosi opstanku osjetljivih otočnih i ruralnih zajednica kroz stalno zapošljavanje tijekom cijele godine (Fezzardi i sur., 2013). Proizvodnja je dominantno usmjerena na uzgoj komarče i lubina te je 2018. godine iznosila 155780 t, s Grčkom (66,25 %) i Španjolskom (21,7 %) kao vodećim državama u proizvodnji. Većina mediteranske uzgojne bijele ribe prodaje se u svježem i neobrađenom obliku manje veličinske kategorije od 300 do 400 grama. Međutim, proizvođači sve više pristupaju vertikalnoj konsolidaciji[3] kako bi postigli ekonomsku opravdanost kod produženog uzgojnog ciklusa. Takav pristup omogućava proširenje proizvodnog asortimana i vrijednost proizvoda kroz procese obrade i prerade jedinki većih masenih kategorija (Barazi-Yeroulanos, 2010; Hadelan i sur., 2012).

Nepovoljno stanje ribolovnih resursa i sve veća potražnja za akvatičkim proizvodima potakli su Europsku komisiju (EK) na strateško opredjeljenje razvoja održivije i konkurentnije akvakulture. Smjernice usvojene u 2021. godini uključuju nekoliko osnovnih i međusobno povezanih ciljeva: (i) izgradnja otpornosti i konkurentnosti; (ii) sudjelovanje u zelenoj tranziciji; (iii) prihvaćenost u društvu i informiranju potrošača i (iv) povećanje znanja i inovacija (Europska komisija, 2021). Poseban naglasak usmjeren je na razvoj i provedbu

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[2] EUMOFA (eng. *European Market Observatory for Fisheries and Aquaculture Products*) – Europski tržišni opservatorij za proizvode ribarstva i akvakulture.

[3] Vertikalna konsolidacija podrazumijeva spajanje prethodno odvojenih jedinica u proizvodnom lancu (npr. mrijestilište, uzgoj, prerada, maloprodaja) u jednu veću cjelinu.

strategije „Od polja do stola“ kao sastavnice „Europskog zelenog plana", koja obuhvaća ključne sastavnice vrijednosnog lanca akvakulture, od proizvodnje, upravljanja i rukovanja proizvodima do prerade, okrupnjivanja ponude i distribucije, tržišta i potražnje potrošača.

Iako je Republika Hrvatska bila pionir u uzgoju najznačajnijih vrsta riba na Mediteranu, rast proizvodnje sporiji je nego u zemljama koji su regionalni predvodnici akvakulturnog sektora. Trenutno se u Hrvatskoj uzgaja deset različitih morskih vrsta na ukupnoj površini od 7,4 km^2, s proizvodnom biomasom u 2019. godini od 17322 tona (Tablica 2). Uzgoj se odvija u svih sedam obalnih županija na gotovo 400 lokacija. Na većini se lokacija provodi uzgoj školjkaša čija su uzgajališta najbrojnija na području Malostonskog zaljeva, dok se uzgoj ribe odvija na oko 50 lokacija, s najvećim brojem u Zadarskoj županiji. Glavnina uzgoja morskih organizama u 2019. godini odnosila se na proizvodnju 6098 t lubina, 6774 t komarča, 2707 t plavoperajne tune i 0,72 t hame (*Argyrosomus regius*, Asso 1801). U proizvodnji dvoljušturnih školjkaša glavnina proizvodnje uključivala je 945 t mediteranske dagnje i 81 t europske plosnate kamenice (*Ostreae edulis*, Linnaeus, 1758).

Tablica 2. Prikaz marikulturnog sektora Republike Hrvatske
(Izvor: Ministarstvo poljoprivrede-Uprava ribarstva).

Pregled uzgojnih površina po županijama u 2019.			
Županije	Bijela riba (m^2)	Tune (m^2)	Školjkaši (m^2)
Dubrovačko – neretvanska	224 890,57	/	1 520 581,95
Istarska	124 560,00	/	850 841,00
Primorsko – goranska	265 412,00	/	42 158,00
Splitsko – dalmatinska	116 977,41	119 600,00	45 440,41
Šibensko – kninska	47 137,75	179 200,00	272 950,00
Zadarska	2 566 263,66	820 000,00	1 520 581,95
Ukupno	3 345 241,39	1 118 800,00	2 963 763,36

Prikaz marikulturne proizvodnje po vrstama u kg						
Vrsta	2014.	2015.	2016.	2017.	2018.	2019.
Lubin	3 214 721	4 488 388	5 310 025	5 615 808	6 220 125	6 089 084
Komarča	3 654 575	4 074 788	4 100 956	4 829 601	5 590 969	6 774 425
Hama	60 063	66 664	125 276	253 338	807 787	724 733
Zubatac	7 356	4 135	1 106	0	0	0
Kalifornijska pastrva	12 779	0	0	0	0	0
Romb	504	7 133	552	0	0	0
Pagar	40 186	0	0	0	0	0
Tuna	2 223 759	2 603 361	2 934 276	2 161 608	3 227 135	2 707 511
Dagnja	713 841	746 382	698 700	919 763	881 627	945 809
Kamenice	32 164	52 079	63 879	62 047	53 756	81 000
Jakobljeva kapica	40	16	66	44	59	16
Ukupno tona	9 959,99	12 042,95	13 234,84	13 842,21	16 781,46	17 322,58

Razvoj nacionalne akvakulture tijekom razdoblja 2014.–2020. bio je potaknut strateškim smjernicama Nacionalnog strateškog plana razdoblja akvakulture (NSPA) u kojem je ukupni planirani proizvodni kapacitet morskih vrsta od 19050 t ostvaren, prvenstveno zahvaljujući uzgoju bijele ribe poput lubina i komarče.

Proizvodnja u sektoru marikulture pokazuje stabilan trend rasta te su određena odstupanja prvenstveno uvjetovana promjenama u ulovnim kvotama tuna. Naime, u svrhu racionalnog gospodarenja i zaštite plavoperajne tune, države članice Međunarodne komisije za očuvanje i zaštitu atlantskih tuna (ICCAT) aktivno sudjeluju u donošenju i provođenju mjera zaštite atlantskih tuna, uključujući provedbu mjera koje se odnose na dozvoljenu minimalnu ulovnu veličinu, razdoblja zabrane ribolova, ulovne kvote te različite mjere kontrole ulova. Hrvatska je članica ICCAT-a od 1997. godine. Kako bi vrsta imala mogućnost mrijesta barem jednom u životnom ciklusu, ICCAT je propisao minimalnu ulovnu masu tune od 30 kg. Međutim, napravljena je iznimka za ulov tuna na području Jadranskog mora budući da je jadranski bazen prepoznat kao hranilište i rastilište mlađih razvojnih stadija plavoperajne tune te je dopušten ulov tune minimalne mase od 8 kg isključivo u svrhu daljnjeg uzgoja, ali dopuštena minimalna masa za stavljanje na tržište je 30 kg (ICCAT, 2006). Stoga uzgojna praksa plavoperajne tune u RH podrazumijeva kavezni uzgoj nedoraslih jedinki u trajanju od dvije do tri godine, dok ne postignu tržišnu veličinu od 30 do 50 kg (Mylonas i sur., 2010). U ostalim mediteranskim državama, uzgojni ciklus tuna traje znatno kraće (do godinu dana) te je imenovan kao tov tuna[4] (Ottolenghi i sur., 2004). Proizvodnja tuna u RH iznosila je 561 t u 2019. godini (Grubišić, 2021) te je zbog uspješnog dugogodišnjeg programa oporavka stoka plavoperajne tune ICCAT posljednjih godina odobrio povećanje izlovnih kvota za 20 %, što pogoduje rastu proizvodnje.

Ukupna proizvodnja bijele ribe od 16781 t u 2018. premašila je proizvodni cilj NSPA od 10000 t do 2020. godine. S obzirom na sve veću potražnju na domaćem i EU tržištu, ali i povećanim investicijama i ulaganjima u modernizaciji postojećih i otvaranju novih uzgajališta, očekuje se i daljnji rast uzgojnih kapaciteta komarče i lubina (Valić i sur., 2012).

Uzgoj školjkaša, osobito kamenica i dagnji, tradicionalan je na hrvatskim prostorima gdje se proizvodnja obavlja na pergolarima u posebno kontroliranim područjima kao što su zapadna obala Istre, Novigradsko more, Velebitski kanal, ušće rijeke Krke, Malostonski zaljev i Malo more. Uzgoj školjkaša, usprkos visokim potencijalnim prinosima, i dalje pokazuje nisku

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[4] ICCAT je definirao uzgoj kao proces držanja nedoraslih jedinki u kavezima u periodu duljem od jedne godine s ciljem višestrukog povećanja biomase, dok se termin tova tuna odnosi na ulovljene adultne jedinke koje se podvrgavaju intenzivnoj ishrani u plutajućim kavezima u periodu od 4 do 6 mjeseci, s ciljem zadovoljavanja specifičnih potreba *sushi* i *sashimi* tržišta (ICCAT, 2007).

proizvodnu stopu. Najveći pad proizvodnje zamijećen je na malostonskim uzgajalištima kao posljedica gubitaka školjkaša zbog ribljih predatora, prvenstveno komarče te zbog nedovoljne dostupnosti ranih razvojnih stadija školjkaša za potrebe nasađivanja na uzgojnim parkovima (Glamuzina i sur., 2014).

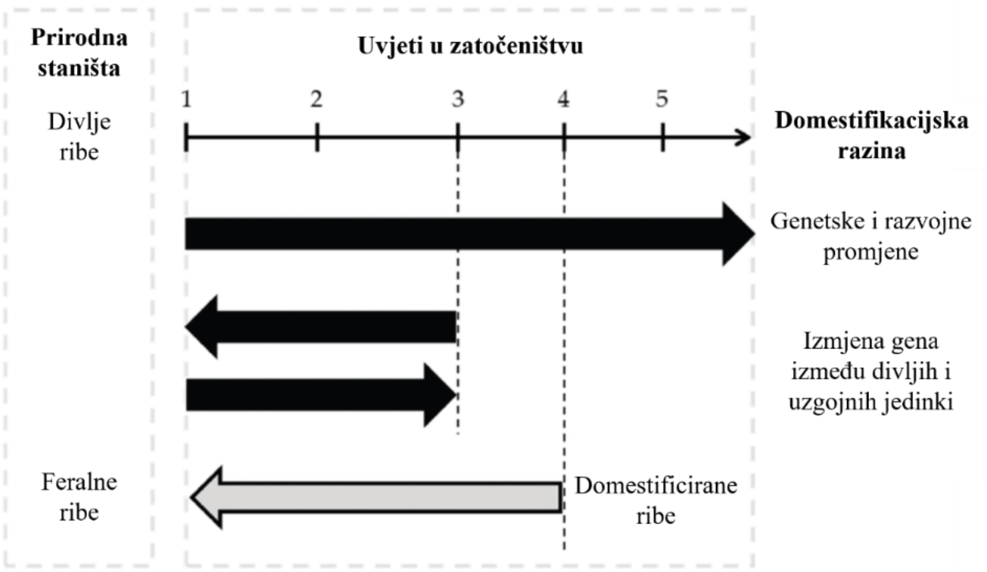
Domestifikacija riba

Domestifikacija je proces uspostave kontroliranog okoliša gdje organizmi tijekom vremena prolaze kroz genetske i razvojne promjene kako bi se prilagodili uzgojnim uvjetima sredine (Lorenzen i sur., 2012). U akvakulturi provodi se kroz prilagodbu vrsta u zatočeništvu gdje su oblik, volumen te hidrodinamičnost uzgojne jedinice, gustoća nasada te dostupnost hrane određeni mjestom i tehnologijom uzgoja, kao i razinom kontrole nad uzgojnim ciklusom vrste (Tablica 3). Teletchea i Fontaine (2014) predložili su model vrednovanja domestifikacije ribljih vrsta na pet razina (Slika 1), u kojem proizvođači kroz postepenu provedbu kontrole nad životnim ciklusom prvo ovladavaju reproduktivnom biologijom vrste i uzgojem ranih razvojnih stadija, prije nego se u potpunosti domestificiraju kroz selekcijske programe.

Tablica 3. Tipovi uzgoja ribljih vrsta prema mjestu, tehnologiji te kontroli uzgojnog procesa.

<table>
<tr><th colspan="12">Mjesto uzgoja</th></tr>
<tr><td colspan="3">Kopno</td><td colspan="3">Riječni estuariji</td><td colspan="3">Obalno more</td><td colspan="3">Otvoreno more</td></tr>
<tr><td colspan="3"></td><td colspan="3"></td><td colspan="3">Zaštićene lokacije
Izložene lokacije</td><td colspan="3"></td></tr>
<tr><th colspan="12">Tehnologija uzgoja</th></tr>
<tr><td colspan="4">Kopnena postrojenja</td><td colspan="4">Bazeni</td><td colspan="4">Kavezi</td></tr>
<tr><td colspan="4">S protočnom izmjenom vode</td><td colspan="4">Zemljani (ukopani) bazeni</td><td colspan="4">Čvrsti kavezi</td></tr>
<tr><td colspan="4">S recirkulacijom vode</td><td colspan="4">Čvrsti bazeni od betona, polietilena, aluminija</td><td colspan="4">Mrežni kavezi na plutajućim i potopivim platformama te na dnu</td></tr>
<tr><th colspan="12">Kontrola životnog ciklusa</th></tr>
<tr><td colspan="6">Uzgoj prikupljenih ili ulovljenih organizama do tržišne veličine</td><td colspan="6">Uzgoj od kontrole matičnog jata, preko mrijesta do tržište veličine</td></tr>
<tr><th colspan="12">Kontrola hranidbe i ulova</th></tr>
<tr><td colspan="6">Intenzivni uzgoj odnosi si se na potpunu kontrolu nasada, hranidbe i izlova</td><td colspan="6">Poluintenzivni i ekstenzivni uzgoj podrazumijevaju djelomičnu kontrolu uz dohraninjivanje ili bez dohranjivanja. Primjenjuje se na zemljištima ili lagunama niske upotrebljivosti za druge djelatnosti.</td></tr>
</table>

Prve tri razine predstavljaju predomestifikacijsku kategoriju jer višegodišnji mrijest nije moguće osigurati, kao ni zadovoljavajuću stopu preživljavanja ranih razvojnih stadija. Akvakultura temeljena na ribolovu spada u spomenutu kategoriju jer životni ciklusi vrsta u zatočeništvu nisu u potpunosti zatvoreni te se nedorasli stadiji love za potrebe uzgoja, kao što je slučaj kod plavoperajne tune. Unos divljih riba prilikom mrijesta i dalje je potreban kako bi se izbjeglo križanje u bliskom srodstvu te održala genetska raznolikost vrsta kod kojih se provodi plansko unošenje riba u ribolovne vode na temelju plana upravljanja. Ribe se smatraju domestificiranim kada čovjek u cijelosti uspostavi kontrolu nad životnim ciklusom vrste u zatočeništvu, od mrijesta do uzgoja proizvoda tržišne veličine (razina 4). Od ukupno 65 morskih vrsta riba koje se uzgajaju u svijetu, za pet je vrsta zatvoren cijeli životni ciklus u zatočeništvu, dok su selekcijski programi u europskoj marikulturi (razina 5) uspješno provedeni kod komarče, lubina i romba (*Psetta maxima,* Linnaeus 1758) (Teletchea, 2015).



Slika 1. Tijek domestifikacije ribljih vrsta kroz razvojne i genetske promjene u zatočeništvu pri čemu broj razine podrazumijeva: 1. prvi pokušaji prilagodbe u zatočeništvu; 2. životni ciklus je dijelom zatvoren u zatočeništvu, ali postoje prepreke u uspostavi kontrole nad reproduktivnom biologijom i uzgojem ranih razvojnih stadija vrste; 3. cijeli je životni ciklus zatvoren u zatočeništvu, ali je potreban unos divljih jedinki kako bi se izbjeglo križanje u bliskom srodstvu; 4. cijeli je životni ciklus zatvoren u zatočeništvu te unos divljih jedinki više nije potreban; 5. uspostava selekcijskih programa na ciljana fenotipska obilježja. Prebjegle uzgojne jedinke u prirodnom staništu izložene su feralizaciji, koja suprotno od domestifikacije, usmjerava genetske i razvojne promjene prema fenotipskim obilježjima svojstvenim divljim ribama (Izvor: Teletchea, 2017).

Uzgoj bijele ribe podrazumijeva zatvoreni ciklus koji započinje u mrijestilištu, a nastavlja se u plutajućim mrežnim kavezima u moru. U mrijestilištu se provodi kontrolirani mrijest i uzgoj ranih razvojnih stadija gdje je ishrana u prva tri tjedna temeljena na živoj hrani (kopepodi i rotifere) nakon čega se uvodi umjetna peletirana hrana do kraja uzgojnog ciklusa. Pored uzgoja osjetljivih razvojnih stadija u mrijestilištu se, prethodno mrijestu, kondicionira matično jato koje je višegodišnjom selekcijom na poželjna fenotipska[5] svojstva izabrano za proizvodnju novih generacija mlađi (Tablica 4). Stopa rasta najčešće je ciljano obilježje jer brži rast skraćuje trajanje uzgojnog ciklusa (Janssen i sur., 2016), dok odabir morfoloških karakteristika poprima sve veću važnost u selekcijskim programima. Proizvod iz akvakulture bolje je prihvaćen i više cijenjen od potrošača ako izgled i kvaliteta proizvoda zadrže sličnost s izvornom sirovinom odnosno ribom iz divljine (Pavlidis i sur., 2011). Preferencijski stavovi o odabiru ribarstvenih proizvoda pokazali su da su, uz cijenu, glavni aspekti odabira kupnje europskih i hrvatskih potrošača izgled i podrijetlo proizvoda (Slika 2). Istraživanje konzumacije ribe u Republici Hrvatskoj ukazalo je da većina potrošača smatra uzgojnu ribu preskupim proizvodom koji nema prirodne karakteristike kao što su okus, boja ili oblik. Riba iz uzgoja često je izložena različitim stereotipovima koji su stečeni neznanjem, s obzirom na to da se gotovo polovica hrvatskih ispitanika izjasnila da ne zna dovoljno o njenim obilježjima, što na kraju utječe na percepciju takvog proizvoda među potrošačima koji nerijetko diskriminiraju ribu iz uzgoja u odnosu na divlju ribu (Eurofish, 2017).

Tablica 4. Selekcijski programi u europskim mrijestilištima na poželjna fenotipska svojstva (Izvor: Chavanne i sur., 2016).

Selektirana obilježja	Lubin	Komarča	Romb
Stopa rasta	●*	●*	●*
Morfologija	●*	●*	
Otpornost na bolesti	●	●	●
Kvaliteta proizvoda	●	●	
Randman	●		
Hranidbena učinkovitost	●	●	
Proizvodnja selektirane mlađi u europskim mrijestilištima			
Proizvodnja mlađi (u milijunima)	525	606	20
Selektirana mlađ (min-max) (%)	34-43	31-44	100

* Najčešće ciljana obilježja selekcijskih programa.

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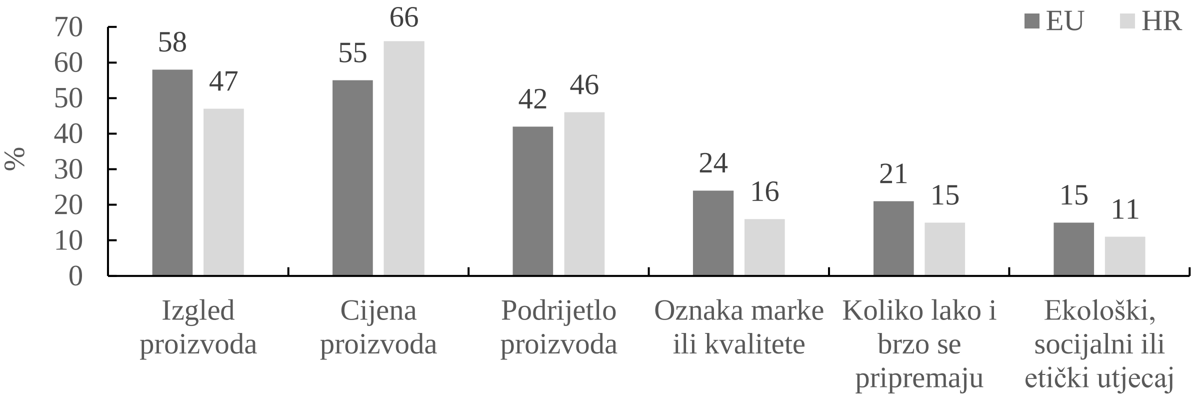
[5] *Fenotip* (grč. *phaino* – predstaviti, *typos* – trag ili lik) podrazumijeva izgled nekog organizma, zbroj svih njegovih obilježja kojima su obuhvaćene sve vanjske i unutarnje strukture i funkcije organizma.



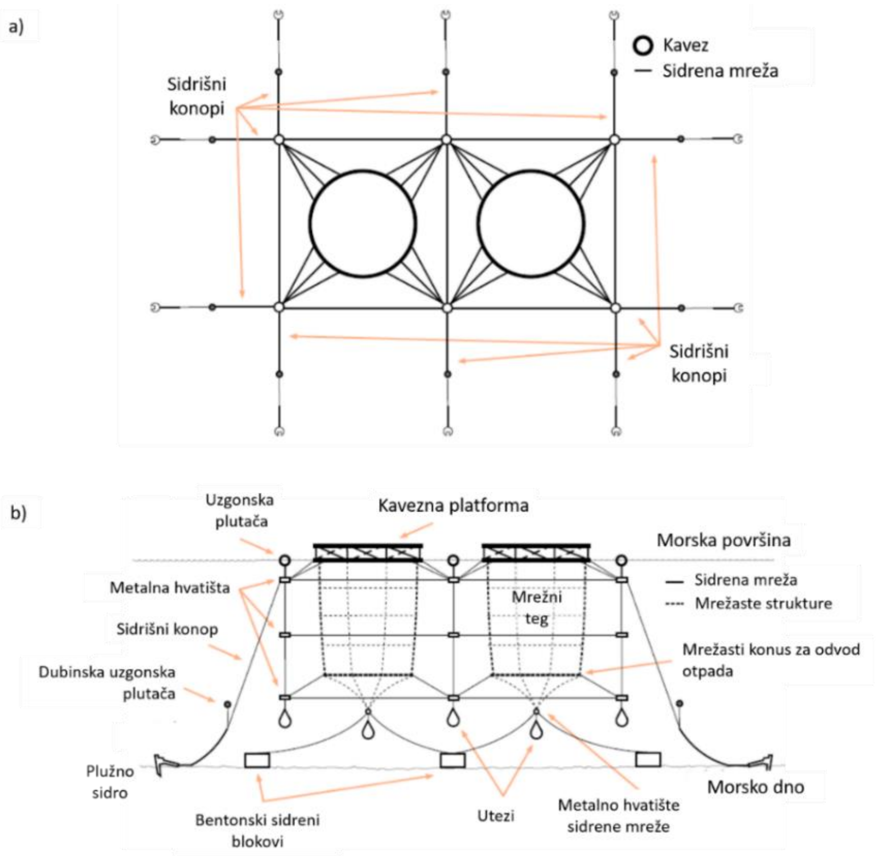
Slika 2. Preferencije potrošača i najvažniji kriteriji za odabir proizvoda iz ribarstva ili akvakulture (Izvor: European Union, 2017).

Ribe starosti od nekoliko mjeseci i mase od 5 do 20 g prenose se u mrežne kavezne sustave na daljnji uzgoj. Standardni uzgojni ciklus traje od 18 do 24 mjeseca kada riba ostvaruje komercijalnu veličinu od 300 do 400 g, iako može varirati u ovisnosti o lokaciji, temperaturi i sastavu hrane, ali i o strategiji proizvođača i potrebama tržišta, pri čemu se krajnja izlovna veličina kreće u rasponu od 250 g do 1,5 kg (Bavčević, 2014).

Uzgoj morskih vrsta riba u mrežnim kaveznim sustavima

Suvremeni kavezni uzgoj predstavlja tehnologiju proizvodnje tržišno najcjenjenijih ribljih vrsta, ali ujedno i proizvodnju s izrazitim okolišnim rizikom. Naime, kavezni uzgoj kao integriran sustav u morskom okolišu može negativno utjecati na kvalitetu morske vode, biološku raznolikost i krajobraz zbog prekoračenja nosivog kapaciteta područja i visokog unosa organske tvari u okoliš, lošeg multisektoralnog planiranja i rukovođenja same djelatnosti te ranjivosti na ekstremne vremenske neprilike (Carballeira Brana i sur., 2020).

Tehničke karakteristike kaveznih sustava. Postavljanje suvremenih uzgojnih postrojenja u morskom okolišu uglavnom se obavlja kroz uspostavu sidrene mreže koja povezuje plutajuće kaveze u dva paralelna niza gdje platforma, mrežni teg te sidrena armatura čine osnovne strukturalne elemente uzgajališta (Slika 3). Platforme na ribogojilištima djeluju kao uzgonsko tijelo budući da se u suvremenoj proizvodni najčešće izrađuju od polietilenskih cijevi visoke gustoće (HDPE) na kojima se vezuje mrežni teg preko okomito postavljenih nosača (Cardia i Lovatelli, 2015). Pravilna raširenost kaveza ostvaruje se potapanjem mrežnoga tega pomoću utega koji se vezuju na dnu kaveza gdje završavaju okomiti konopi koji povezuju mrežni teg s platformom. Sidrena armatura sastoji se od (i) sidara, u obliku plužnog sidra i/ili betonskih blokova; (ii) sidrine, koju najčešće čine pocinčani lanci i poliamidni konopi te (iii) sidrene mreže, gdje se svaki usidreni kavez nalazi u svom polju (Cardia i Lovatelli, 2015). Metalna hvatišta, u obliku ploča ili prstena, predstavljaju čvorišta sidrenih polja preko kojih se povezuju svi elementi sidrene armature. Sidrena polja povezana su sa sidrinama na vanjskim hvatištima plutača što omogućuje svakom stacionarnom kavezu lagano pomicanje unutar svog polja ovisno o smjeru djelovanja vjetra i morskih strujanja, čime se smanjuje vjerojatnost pojavnosti havarija na uzgojnim koncesijama (Bavčević, 2014; Cardia i Lovatelli, 2015).



Slika 3. Shematski prikaz tipičnog postavljanja sidrenog polja iz a) zračnog pogleda i b) bočnog presjeka, s naznačenim plutajućim i potopljenim strukturama ribogojilišta (Izvor: Sosa i sur., 2020).

Odabir uzgojnih lokacija. Prostorno planiranje marikulture, uključujući zonaciju te smještaj sadržaja u prostor, ima cilj omogućiti održivo korištenje morskog prostora za djelatnost uzgoja. Ovakav integralni pristup upravljanja obalnim područjem podržava proizvodnju, smanjuje mogućnost sukoba s drugim korisnicima i namjenama te održava integritet okoliša (Sanchez-Jerez i sur., 2016; Katavić, 2017). Prilikom ovakvog pristupa planiranju koriste se multikriterijalne analize cjelokupnog prostora s ciljem izdvajanja zona ili lokacija u skladu s važećim propisima za uzgoj ribe (Tablice 5 i 6). Definiranje povoljnog morskog okoliša za uzgoj ribe, s naglaskom na klimatske uvjete, od velike je važnosti za odabir konstrukcijsko-tehničkih svojstava kaveznih instalacija (Cardia i Lovatelli, 2015). U skladu s prevladavajućim meteooceanografskim uvjetima i dubinom utvrđuje se stabilnost sidrenja plutajućih kaveznih instalacija kako bi se potencijalne havarije svele na najmanju mjeru jer gubitak ribe i opreme predstavlja financijski teret za uzgajivača, ali i potencijalan rizik za okoliš zbog moguće interakcije između divlje i uzgojne ribe (Jackson i sur., 2015).

Tablica 5. Kriteriji pogodnosti dijelova pomorskog dobra za kavezni uzgoj bijele ribe (Izvor: Narodne novine , 2012).

Kriterij	Dobro	Srednje	Loše
Valovi	1 -3 m	< 1 m	> 3 m
Dubina	> 3 visine mrežnoga tega	2 -3 visine mrežnoga tega	< 2 visine mrežnoga tega
Strujanja	>10 cm/s	5 – 10 cm/s	< 5 cm/s
Otopljeni kisik (%)	> 90	70 – 90	< 70
Temperatura mora (°C)	22 – 25	25 – 27	> 27; < 8
Slanost (‰)	> 25	15 – 25	< 15
Struktura i sastav sedimenta	Pjeskovito /šljunkovito	Hridinasto	Muljevito
Trofički status	Oligotrofno	Mezotrofno	Eutrofno
Bentos	Nizak	Umjeren	Razvijen
Predatori	Nema	Rijetki	Učestali

Tablica 6. Kriteriji pogodnosti dijelova pomorskog dobra za kavezni uzgoj tuna (Izvor: Narodne novine, 2012).

Kriterij	Dobro	Loše
Izloženost otvorenom moru	Otvoreno/poluotvoreno	Uvale/zaljevi
Dubina	> 50 m	< 50 m
Strujanja	10 cm/s	< 10 cm/s
Slanost (‰)	36 - 39	< 36 i > 39
Otopljeni kisik (%)	> 90	< 90
Trofički status	Oligotrofno	Eutrofno
Fitobentos (livade morskih cvjetnica)	Na dnu nema livada morskih cvjetnica ili su na sigurnoj udaljenosti koju određuje postupak procjene utjecaja na okoliš	Na dnu ili u blizini lokacije nalaze se livade morskih cvjetnica

Hrana i hranidba riba. Hranidba je važan tehnoloških postupak kod kaveznog uzgoja jer utječe na troškove proizvodnje (40–60 % troškova), rast i zdravlje organizma u uzgoju te kvalitetu samog proizvoda. Općenito, riblja hrana formulirana je u skladu s potrebama riba, preporukama nutricionista i uzgajivača riba. Najčešća krmiva koja se rabe za proizvodnju ribljih krmnih smjesa su: riblje brašno, brašno različitih žitarica, nusproizvodi industrije škroba te vitaminsko-mineralni premiksi (Pavličević, 2016). Kao i za većinu uzgojnih karnivornih morskih riba, glavni sastojci hranjiva za kavezni uzgoj lubina i komarče na istočnoj strani Jadrana jesu riblje brašno i ulje, dok se tune hrane svježom ili zamrznutom malom plavom ribom.

Akvakultura je glavni korisnik ribljih brašna i ulja u stočarskoj industriji čija potrošnja premašuje 70 % od ukupne svjetske proizvodnje (IFFO[6], 2021). Riblje brašno sadrži vrijedne visokoprobavljive bjelančevine, kao i bitne vitamine i minerale, dok je riblje ulje vrijedan izvor lipida, kao što su korisne omega (ω)-3 polinezasićene masne kiseline (PUFA) (Turchini i sur., 2010). Međutim, zbog trenda rasta uzgoja akvatičkih organizama i smanjenja opskrbe iz ulova ribe u svjetskim morima, potražnja za bjelančevinama u ribljoj hrani vjerojatno će premašiti godišnju svjetsku opskrbu ribljim brašnom (Matulić i sur., 2011). Stoga je pronalaženje odgovarajućih sastojaka za zamjenu ograničenih morskih resursa, koji se uobičajeno koriste u hrani za akvakulturu, jedan od najvažnijih izazova u sektoru. Glavna je prepreka pronaći alternativne izvore koji su hranjivi kao riblje ulje (Hixson i sur., 2013). Morskim je ribama potrebna arahidonska kiselina (ARA, 20:4ω6) i visoka razina esencijalnih omega-3 masnih kiselina, eikosapentaenska kiselina (EPA, 20:5ω3) i dokosaheksaenska kiselina (DHA, 22: 6ω3) jer ih same ne mogu lako sintetizirati. Također, hranidba ribe u kaveznoj fazi uzgoja financijski znatno opterećuje proizvodnju zbog čega se u ribljoj hrani sve veći udio ribljeg brašna i ulja zamjenjuje jeftinijim sirovinama kopnenog podrijetla. Od biljnih sastojaka prevladavaju uljarice kao što su soja i uljana repica, dok su iz mesne industrije dozvoljeni samo pojedini tipovi sirovina poput krvnog brašna ili brašna od nusproizvoda prerade mesa od peradi (Boyd i sur., 2020; Naylor i sur., 2021).

Hranidba kavezne ribe tijekom posljednjih dvaju desetljeća znatno je unaprijeđena zbog novijih tehnologija prerade sirovina u ekstrudirani[7] pelet, poboljšanja hranidbene učinkovitosti kroz selekcijske programe te upotrebe alternativnih sirovina pri proizvodnji riblje hrane (Boyd i sur., 2020; Naylor i sur., 2021). Zamjena ribljeg brašna izvorima bjelančevina biljnog i

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[6] IFFO (eng. *International fishmeal and fish oil organization*) - Međunarodna organizacija za riblje brašno i riblje ulje.

[7] Ekstruzija omogućava povećanje unosa energije mastima što smanjuje relativnu potrebu za bjelančevinama čime je postignuto dodatno smanjenje emisije nutrijenata.

životinjskog podrijetla doprinijela je razvoju jeftinije riblje hrane, a time i većem broju morskih vrsta riba koje se uzgajaju isključivo na krmivima (Tablica 7). Selekcija na stopu rasta i učinkovitiju probavu bjelančevina kopnenog podrijetla omogućili su manju potrošnju hrane za ostvarivanje prirasta od 1 kg, što je doprinijelo ekonomskoj, ali i ekološkoj održivosti uzgoja ribljih vrsta zbog smanjenog unosa organske tvari u morski okoliš (Naylor i sur., 2021).

Tablica 7. Parametri učinkovitosti hranidbe riba u akvakulturi (Izvor: Naylor i sur., 2021).

	Salmonidi		Morske vrste riba	
	1997	2017	1997	2017
Ukupna proizvodnja (u 1000 t)	741	2577	646	3098
%-tni udio riba uzgojen na krmivu	100	100	53	82
Faktor konverzije hrane (FCR)[8]	1,4	1,3	2	1,7
%-tni udio ribljeg brašna u krmivu	43	12	50	14
%-tni udio ribljeg ulja u krmivu	25	10	15	5

U odnosu na druge akvakulture vrste, negativna percepcija potrošača prema uzgojnim tunama hranjenim peletiranom hranom stvara otpor kod uzgajivača da prihvate nove hranidbene strategije koje uključuju pelete (Grigorakis i Rigos, 2010). Hranidba tuna obavlja se putem cjelovite male plave ribe (eng. *baitfish*) poput srdele (*Sardina pilchardus*, Walbaum 1792) te haringe (*Clupea harengus*, Linnaeus 1758), ali i drugih vrsta kao što su lokarde (*Scomber japonicus*, Houttuyn 1782), bukve (*Boops boops*, Linnaeus 1758), šnjura (*Trachurus* sp.) i lignji (*Illex* sp.) (Ottolenhgi, 2008), čiji je cilj značajno povećati sadržaj masnog udjela tkiva za japanska *sushi* i *sashimi* tržišta (Mylonas i sur., 2010). Većina hrvatskih uzgajivača koristi veliki udio haringe u hranidbi tuna jer sadrži veći postotak lipida u odnosu na druge izvore, ali i zbog činjenice da se dostatne količine male plave ribe ne mogu osigurati na lokalnom tržištu. Tuna se obično u toplijem razdoblju godine hrani u iznosu od 5 do 7 %, a u hladnijem razdoblju od 2 do 3 % ukupne biomase, s FCR-om koji se nalazi u omjeru od 15 do 20:1 kod većih te od 10 do 15:1 kod manjih tuna (Bavčević i sur., 2016). Međutim, javlja se sve veća potreba za razvojem ekstrudirane hrane koja bi trebala poduprijeti kontroliraniju hranidbenu strategiju te umanjiti ribolovni pritisak na iskorištavane riblje stokove jer je potrebno osigurati velike količine sirovine za uzgoj i tov tuna. Unatoč tome što je umjetna peletirana hrana za uzgoj tuna proizvedena, spomenuta tržišta ne pokazuju interes za tako uzgojenim proizvodima, prvenstveno zbog utjecaja peletirane riblje hrane na okus sirovog proizvoda (Ottolenghi, 2008).

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[8] Faktor konverzije hrane (FCR) predstavlja biometrijski indeks koji pokazuje kolika je količina hrane potrebna da bi se ostvario prirast organizma u iznosu od 1 kg.

Utjecaj na okoliš. Zbog stalnog rasta broja lokacija za uzgoj morskih organizama javlja se sveprisutna zabrinutost da akvakultura kao gospodarska djelatnost može predstavljati opasnost za morsku biološku raznolikost kroz negativni utjecaj na prirodne populacije morskih organizama kao i cjelokupni morski ekosustav (Tablica 8). Glavni učinci uključuju degradaciju staništa (Claudet i Fraschetti, 2010), zagađenje hranjivim tvarima (Waite i sur., 2014), uvođenje neautohtonih vrsta (Šegvić-Bubić i sur., 2016) te genetske utjecaje i smanjenje adaptivne vrijednosti ili fitnesa (eng. *fitness*) divljih populacija iste vrste (Glover i sur., 2017; Šegvić-Bubić i sur., 2011a, 2014, 2017).

S ciljem razvoja održive akvakulture, unaprijeđena formulacija riblje hrane, provedba zootehničkih praksi i zoniranja pogodnih akvakulturnih područja smanjili su unos nutrijenata u okoliš. Povećanje sadržaja lipida na uštrb bjelančevina u ribljoj hrani te pozicioniranje kaveza u duboka područja s dovoljnim protokom vodenih masa najčešće su korišteni koraci s pozitivnim ishodom za okoliš (Tičina i sur., 2020). U većini ribogojilišta obogaćenje sedimenta dušikom i fosforom minimalna su i ograničena na područja do 500 m od kaveza (Price i Morris, 2013). Međutim, fizikalne i kemijske oscilacije uzgojnog okoliša, kao i sama uzgojna biomasa, potiču i biološke promjene, ne samo kod uzgajanih riba nego i kod organizama u okruženju. Ekološke se interakcije odražavaju kroz činjenicu da kavezne uzgojne instalacije postaju nove ekološke niše za prirodne populacije ribe kod kojih su moguće promjene u fiziologiji i ponašanju. Drugi rizik za okoliš očituje se u mogućem utjecaju zbjegova uzgojne ribe iz kaveza, prisutnih u morskom okolišu kao rezultat slučajnog bijega iz kaveza ili namjernog poribljavanja.

Tablica 8. Utjecaji akvakulture na morski ekosustav (Izvor: Karakassis, 2007).

Izvor pritiska	Mogući učinak na biotu	Pogođene zajednice	Relativna/ očekivana prostorna skala	Vrsta utjecaja	Procjena oporavka zajednice
Fizička konstrukcija	Neposredan mortalitet zbog zaplitanja	Kralježnjaci	lokalna	-	srednji
	Promjene u ponašanju obalne pelagične ribe	Kralježnjaci (riba)	lokalna	?	nepoznat
	Promjene u ponašanju obalnih ptica i morskih sisavaca.	Kralježnjaci	lokalna-mezo	-	nepoznat
Sustav za kontrolu predatora	Neposredan mortalitet	Kralježnjaci	lokalna-mezo	-	nepoznat
	Promjene u ponašanju divlje faune	Kralježnjaci	lokalna-mezo	-	nepoznat
Bježanje ribe	Prijenos bolesti na druge vrste	Raznovrsne (najčešće riba)	mezo-široka	-	nepoznat
	Genetske interakcije s divljom ribom	Kralježnjaci (riba)	mezo-široka	-	spor
	Raseljavanje divlje ribe iz njenog prirodnog staništa (npr. kroz kompeticiju, predaciju)	Kralježnjaci (riba)	mezo-široka	-	nepoznat
Otpuštanje nepojedene hrane	Gušenje i raseljavanje bentičkih organizama	Makrofauna	lokalna	-	spor
	Gubici mrijesta, hranilišta i/ili mrijestilišta divljih vrsta	Raznovrsne	lokalna	-	spor
	Gubitak bioraznolikosti	Makrofauna	lokalna-mezo	-	spor
	Fragmentacija bentičkog staništa	Raznovrsne	lokalna-mezo	-	spor
Otpuštanje nutrijenata	Promjena kvalitete vode	Raznovrsne	lokalna-mezo	-/+	brz
	Mortalitet planktona (uključujući jaja i ličinke riba i beskralježnjaka)	Raznovrsne	lokalna	-	brz
	Povećana primarna produkcija	Raznovrsne	lokalna-mezo	-/+	brz
	Izmjena sastava planktonske zajednice	Fitoplankton	lokalna-mezo	?	brz
	Povećano cvjetanje štetnih algi	Raznovrsne	lokalna-mezo	-	brz
	Propadanje livada morskih trava	Morske biljke i posredno raznovrsne zajednice	lokalna-mezo	-	spor
Antibiotici	Propadanje divljih vrsta	Raznovrsne	lokalna	-	brz
	Promjene u bentičkim bakterijskim zajednicama	Mikrobi	lokalna	-	nepoznat
	Otporne vrste mikroba	Posredno raznovrsne	nepoznata	-	nepoznat
Pesticidi	Neposredan mortalitet i subletalni učinci	Beskralježnjaci	lokalna	-	nepoznat
	Propadanje divljih vrsta	Raznovrsne	lokalna	-	nepoznat
Dezinficijensi i sredstva protiv obraštaja	Neposredan mortalitet i subletalni učinci	Beskralježnjaci	lokalna	-	nepoznat
	Propadanje divljih vrsta	Beskralježnjaci	lokalna-mezo	-	nepoznat
	Promjene u fiziologiji	Beskralježnjaci	lokalna-mezo	-	nepoznat

Prirodne populacije riba u okruženju uzgajališta riba

Fizičke umjetne strukture stvorene čovjekovim djelovanjem oko kojih dolazi do okupljanja morskih vrsta riba različitih ontogenetskih stadija poznate su kao FAD objekti (*engl. Fish Aggregation Devices*). Priobalna i pučinska uzgajališta u vodenom stupcu predstavljaju trodimenzionalna staništa čije plutajuće i potopljene strukture utječu na zadržavanje i strukturu prirodnih ihtionaselja, prvenstveno kroz (i) povećanu dostupnost trofičkih resursa u obliku obraštajnih zajednica; (ii) prisutnost zaklona, čime se smanjuju stope predacije ranih razvojnih stadija i pospješuju procesi novačenja te (iii) privlačenja predatornih vrsta riba zbog okupljanja plova riba (Forrest i sur., 2007, Callier i sur., 2018). U Sredozemnom moru aktivna je oko 21000 uzgajališta morskih vrsta riba (Trujillo i sur., 2012), čije koncesijske površine djeluju kao visokoproduktivna staništa priobalnih ihtiozajednica (Sanchez-Jerez i sur., 2011; Bayle-Sempere i sur., 2013). Zbog zabrane ribolova na područjima gdje se odvija uzgoj, lokaliteti kao takvi poistovjećeni su sa zaštićenim morskim područjima gdje odljev velikog broja gospodarsko važnih vrsta, poput onih iz porodica Carangidae, Mugilidae i Sparidae, pozitivno utječe na lokalno ribarstvo, pogotovo u oligotrofnim morima kao što je sredozemni bazen (Dempster i sur., 2006; Arechavala-Lopez i sur., 2010).

Uz poznate načine privlačenja FAD-ova, uzgajališta riba pružaju i cjelogodišnju dostupnost velikim količinama hrane, gdje se u ovisnosti o uzgojnoj vrsti i primijenjenim zootehničkim praksama nepojedeni udio hrane tijekom hranidbe kreće od 8 do 52 % za bijelu ribu (Ballester-Moltó i sur., 2017) te 4 do 23 % za tune (Bruce, 1997). Stalnim priljevom hrane s uzgajališta povećava se brojnost, biomasa i vrijeme zadržavanja populacija oko kaveznih instalacija u odnosu na područja bez uzgoja (Sanchez-Jerez i sur., 2011).

Koncentriranje i zadržavanje uzgojnih organizama na jednome mjestu, uz dodatak hrane koja nije autohtonog trofičkog podrijetla, može uzrokovati promjenu kategorije staništa u stanište s dodanim izvorom energije (Bayle-Sempere i sur., 2013). Nepojedena riblja hrana predstavlja unos dodatnog izvora energije koji utječe na trofičku strukturu lokalnih ihtiozajednica, odnosno mijenja prirodne obrasce ponašanja riba tijekom ishrane (Tuya i sur., 2006; Bayle-Sempere i sur. 2013). Od ukupno 160 zabilježenih ribljih vrsta koje obitavaju u okolici ribogojilišta za njih 17 utvrđena je konzumacija hrane akvakulturnog podrijetla (Sanchez-Jerez i sur., 2011). Učinak privlačenja i okupljanja ribljih vrsta važan je jer doprinosi smanjenju emisija uzgoja na bentoske zajednice s obzirom na to da okolne populacije djeluju poput prirodnog biofiltera koji može ukloniti i do 20 % organskog otpada s ribogojilišta (Ballester-Moltó i sur., 2017). Stoga fitoplanktonsko iskorištavanje nutrijenata te nepojedena hrana koju potroše lokalne divlje ihtiopopulacije sinergijski umanjuju negativan utjecaj organskog otpada na sediment i biocenoze morskog dna (Tičina i sur., 2020).

Prostorno-vremenska varijabilnost raspodjele i brojnosti okupljenih populacija uglavnom je uvjetovana obalnom geografijom, topografijom morskog dna, udaljenošću od obale i raznolikošću staništa u blizini ribljih farmi, kao i sezonskim uvjetima i fenologijom vrste (Dempster i sur., 2005; Ballester-Moltó i sur., 2015). Na području Sredozemnog mora utvrđeno je oko 20 vrsta riba koje se zadržavaju u okolici ribogojilišta bijele ribe tijekom cijele godine, s najvećom pripadnošću obiteljima Sparidae, Mugilidae, Atherinidae i Carangidae (Tablica 9). Slični obrasci u varijabilnosti okupljanja divljih pelagičnih vrsta riba uočeni su i u okolici uzgajališta tuna (Šegvić-Bubić i sur., 2011b; Bacher i sur., 2012). Sastav i brojnost ribljih zajednica uzgajališta tuna pokazuju drukčiju strukturu od one uočene na uzgajalištima lubina i komarče diljem Sredozemnog mora, vjerojatno kao posljedica drukčijeg uzgojnog okoliša (dubina lokacije, strujanja, udaljenost od obale) te načina ishrane (sitna plava riba *vs.* ekstrudirana hrana) (Šegvić-Bubić i sur., 2011b).

Dostupnost ekstrudirane hrane u obliku peleta pozitivno utječe na učestalost hranidbe ribljih populacija u okolici uzgajališta, što doprinosi boljoj tjelesnoj kondiciji, a time i rastu te povećanju reproduktivnog potencijala kavezno povezanih jedinki (Sanchez-Jerez i sur., 2011; Callier i sur., 2018). Međutim, uočen je izmijenjen kvantitativni i kvalitativni sastav masti i masnih kiselina kod divljih riba povezanih s uzgajalištima kao fiziološki odgovor na ishranu koja sadrži riblje pelete (Fernandez-Jover i sur., 2007).

U odnosu na lubina i komarču koji su poikilotermni organizmi, tuna je regionalni heteroterm koji ima mogućnost reguliranja tjelesne temperature pojedinih dijelova tijela što iziskuje veću potrebnu količinu energije za održanje standardnog metabolizma nego što je to slučaj kod bijele ribe (Benetti i sur., 2016). Budući da u uzgojnoj praksi ekstrudirana peletirana hrana još uvijek nije sastavni dio prehrane tuna, potrošnja sirovine (sitna plava riba) je znatno veća od one namijenjene kaveznom uzgoju bijele ribe (Ottolenghi, 2008). Procjena o ukupno utrošenoj hrani za potrebe uzgoja tuna iznosi 94139 t na ostvarenu proizvodnu količinu od 5537 tona za razdoblje od 2015. i 2016. godine (Tablica 2), pri FCR od 15 do 20 kg za kilogram masenog prirasta. Uz pretpostavku gubitka hrane od 10%, nepojedena hrana na hrvatskim tunogojilištima tijekom promatranog perioda dostiže i do 9400 t odnosno 8,4 kg/m^2 koncesijske površine, što predstavlja značajan izvor hrane za okolne morske organizme. Dodatni trofički resursi omogućili su prepoznavanje tunogojilišta kao područja ribljih mrijestilišta, hranilišta i rastilišta gdje su brojne populacije mlađi, spolno nezrelih i zrelih jedinki riba postali njihovi stalni stanovnici (Šegvić-Bubić i sur., 2011b; Žužul i sur., 2019).

Tablica 9. Prikaz najzastupljenijih ribljih vrsta uočenih oko uzgajališta morskih vrsta riba.

Izvor	Uzgojne vrste u kavezima	Vrste sa znatnijom brojnošću u blizini istraživanih ribogojilišta
Akyol i Ceyhan (2020)	Lubin, komarča	*Atherina boyeri, Balistes capriscus, Belone belone, Pseudocaranx dentex, Seriola dumerili, Trachinotus ovatus, Trachurus mediterraneus, Trachurus trachurus, Spicara smaris, Sardina pilchardus, Sardinella aurita, Coryphaena hippurus, Engraulis encrasicolus, Dicentrarchus labrax, Chelon auratus, Chelon labrosus, Chelon saliens, Mugil cephalus, Thunnus thynnus, Boops boops, Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris, Oblada melanura, Sarpa salpa, Sparus aurata*
Dempster i sur., 2005 (JZ Mediteran)	Lubin, komarča	*Seriola dumerilli, Trachinotus ovatus, Trachurus mediterraneus, Sardinella aurita,* Mugilidae, *Pomatomus saltatrix, Boops boops, Oblada melanura, Diplodus vulgaris, Pagellus acarne*
Fernandez-Jover i sur., 2008 (JZ Mediteran)	Lubin, komarča	*Seriola dumerilli, Trachinotus ovatus, Trachurus mediterraneus, Sardinella aurita,* Mugilidae, *Pomatomus saltatrix, Boops boops, Oblada melanura, Diplodus vulgaris, Sarpa salpa, Sparus aurata,*
Fernandez-Jover i sur., 2009 (JZ Mediteran)	Lubin, komarča	*Trachinotus ovatus, Trachurus mediterraneus, Chelon labrosus, Liza aurata, Liza ramada,* Mugilidae, *Pomatomus saltatrix, Boops boops, Oblada melanura, Diplodus vulgaris, Sarpa salpa, Sparus aurata*
Valle i sur., 2007 (JZ Mediteran)	Lubin, komarča	*Seriola dumerilli, Trachurus mediterraneus, Pomatomus saltatrix, Boops, boops, Oblada melanura, Diplodus vulgaris, Sparus aurata*
Bacher i sur., 2012	Tuna	*Balistes capriscus, Belone belone, Seriola dumerili, Trachurus spp., Trachurus ovatus, Prionace glauca, Hyperoglyphe perciformes, Sardinella aurita, Sardina pilchardus, Sarda sarda, Scomber japonicus, Thunnus thynnus, Diplodus vulgaris, Diplodus puntazzo, Oblada melanura, Sarpa salpa*
Šegvić-Bubić i sur., 2011b (istočni Jadran)	Tuna	*Atherina hepsutus, Belone belone, Seriola dumerilli, Trachurus trachurus, Trachurus picturatus, Spicara flexuosa, Spicara maena, Chromis chromis, Sarda sarda, Scomber japonicus, Thunnus thynnus, Boops boops, Diplodus puntazzo, Diplodus sargus, Diplodus vulgaris, Diplodus annularis, Oblada melanura, Sarpa salpa, Sardina pilchardus, Symphodus rostratus, Pagellus acarne*

Riblji zbjegovi iz uzgajališta

Zbjegom se smatraju uzgojne jedinke svih ontogenetskih stadija koje zbog različitih razloga i uvjeta na uzgajalištu pristupaju bijegu iz kaveznih infrastruktura u morski okoliš. Prebjegle ribe prilagođavaju se uvjetima prirodnog staništa kroz proces feralizacije, koji suprotno od domestifikacije, usmjerava genetske i razvojne promjene prema obilježjima svojstvenim divljim ribama. Brzina prilagodbe ovisi o razvojnom stadiju zbjega kao i o domestifikacijskoj razini riblje vrste. Uzgojne jedinke koje potječu iz populacija nad kojima nije u potpunosti uspostavljena kontrola životnog ciklusa (Slika 1) brže poprimaju divlja fenotipska obilježja u odnosu na populacije nad kojima se duži niz generacija vrši selekcija na poželjna fenotipska svojstva (Teletchea, 2017).

Bijeg uzgojnih riba iz uzgajališta predstavlja neizbježnu pojavu u akvakulturnoj praksi koja može proizaći iz ljudske pogreške pri rutinskom rukovanju kaveznim mrežama, mehaničkih kvarova i šteta uzrokovanih nepovoljnim vremenskim uvjetima ili predatorima poput dupina i strijelke koji znaju trgati mrežni teg (Jackson i sur., 2015). Pokazalo se da zbjegovi imaju štetne učinke na prirodne riblje populacije zbog kompeticije, širenja bolesti i promjene genetske raznolikosti uslijed hibridizacije (McGinnity i sur., 2003; Read i Fernandes, 2003). Desetljeća intenzivnog uzgoja i selekcijskih pritisaka na poželjna proizvodna obilježja dovela su do promjena ne samo morfoloških već i genetskih karakteristika uzgojnih u odnosu na divlje populacije (Brown, 2003). Naime, pri masovnoj proizvodnji ranih razvojnih stadija riba najčešće se u komercijalnim mrijestilištima koristi ograničen broj spolno zrelih matica koje su prethodno selekcionirane na važna fenotipska svojstva (Tablica 4). Spomenute aktivnosti u mrijestilištima, gdje izostaje genetski kontrolirani mrijest matica, utječu na gubitak genetske raznolikosti potomaka, s većim udjelom homozigotnih jedinki naspram heterozigotnih, što pogoduje ispoljavaju recesivnih osobina u budućim generacijama. Suprotno scenariju koji pogađa matičnjake, visoka genetska varijabilnost prirodnih populacija održava se zahvaljujući promjenama kao što su migracije gena, mutacije te prirodna selekcija i genetski drift. Stoga potencijalno križanje zbjegova s lokalno-divljim populacijama može značajno utjecati na adaptivnu vrijednost (engl. *fitness*[9]) novonastalih hibridnih jedinki te dovesti do promjena u frekvenciji i uvođenju nenativnih alela u lokalne populacije, dovodeći do poremećaja prilagođenih lokalnih genotipova (Hindar i sur., 2006; Ward, 2006). Time se ugrožava prirodna

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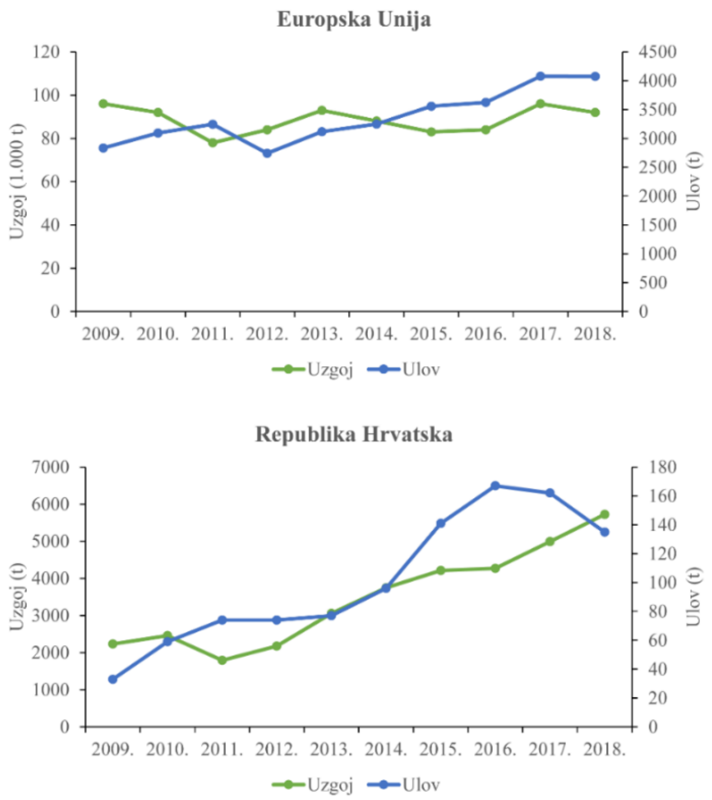
[9] *Adaptivna vrijednost* (eng. *fitness*) opisuje sposobnost preživljavanja i razmnožavanja te je jednak prosječnom doprinosu genskom fondu naredne generacije koji je strukturiran prema prosjeku jedinki određenog genotipa ili fenotipa.

genetska varijabilnost vrste koja je nužna za uspješno prilagođavanje okolišnim promjenama i njeno preživljavanje.

Čimbenici koji najviše doprinose utjecaju zbjegova na ihtiopopulacije u blizini kaveza su (i) stopa prebjega; (ii) opseg selekcije osobina uzgojnog stoka; (iii) sposobnost preživljavanja i reprodukcije prebjega u prirodnoj okolini, koja je određena ishranom i međusobnim križanjem s divljim jedinkama; (iv) veličinom, distribucijom i zdravstvenim statusom ihtiopopulacije te (v) ukupna dužina ribe pri izlovu (Forrest i sur., 2007: Arechavala-Lopez i sur., 2017). Potaknuti promjenama u navikama potrošača, gdje je sve veće zanimanje za hranu koja ne zahtijeva dugu pripremu, proizvođači produljenjem uzgojnog ciklusa do 40 mjeseci nastoje ostvariti masene raspone jedinki koje premašuju 500 g. Time im je omogućena lakša obrada i prerada primarne sirovine u polugotove i gotove proizvode (Dimitriou i sur., 2007; Barazi-Yeroulanos, 2010). Međutim, produljeni uzgojni ciklus omogućuje spolno sazrijevanje većine uzgojnih vrsta i pojave "zbjega putem mrijesta" riba u zatočeništvu, gdje se otpuštaju spolne stanice i oplođene jajne stanice iz kaveznih infrastruktura u okolno more (Dimitriou i sur. 2007; Jensen i sur. 2010; Somarakis i sur., 2013). No, spontani mrijest tijekom uzgojnih aktivnosti u kavezima može imati i pozitivne učinke na morsku biotu kroz doprinos povećanju brojnosti populacija pod intenzivnim ribolovnim pritiskom, što je opaženo za plavoperajnu tunu u uzgoju na istočnoj strani Jadrana, vrstu od interesa za akvakulturu temeljenu na ribolovu (Džoić i sur., 2017; Tičina i sur., 2020)

Razvoj industrije kaveznog uzgoja lubina i komarče u EU i RH pospješuju zabrinutost o potencijalnim utjecajima zbjega na autohtone populacije (Šegvić-Bubić i sur., 2011a; Arechavala-Lopez i sur., 2017). U zadnjem je desetljeću uočen porast ulova divlje komarče (Slika 4). Međutim, paralelno s povećanjem ulova, zabilježen je i značajan porast proizvodnje komarče u uzgoju i porast uzgojnih kapaciteta što pogoduje većem broju zbjegova te doprinosi povećanju ribolovnog ulova vrste (Glamuzina i sur., 2014). Prebjezi lubina i komarči imaju sposobnost plivanja i nekoliko kilometara, udaljavajući se od matičnog uzgajališta te boravka na susjednim uzgojnim koncesijama i obalnim ribolovnim područjima (Arechavala-Lopez i sur., 2011; 2012b). Praćenjem bijele ribe iz uzgoja telemetrijskim metodama uočena je visoka stopa preživljavanja nakon bijega iz kaveza te zadržavanje jedinki i po nekoliko mjeseci u okolici uzgajališta (Toledo-Guedes i sur., 2009; Arechavala-Lopez i sur., 2011, 2012b; Šegvić-Bubić i sur., 2018). Posljedično, sve su prisutnija negativna međudjelovanja uzgojnih i autohtonih populacija kroz kompeticiju za resurse, predaciju i izmjenu genetske strukture potomaka kroz međusobno križanje (Glamuzina i sur., 2014; Šegvić-Bubić i sur., 2011a; 2014; Žužul i sur., 2019).



Slika 4. Odnos uzgoja i ulova komarče tijekom razdoblja od 2009. do 2018. godine (Izvor: Državni zavod za statistiku, EUMOFA).

Brojnije prisustvo komarče u morskom okolišu, uvjetovano zbjegovima iz akvakulture, povoljnim ekološkim uvjetima i/ili klimatskim promjenama, izravno utječe na održivost proizvodnje školjkaša. Naime, predacija komarče na uzgajalištima školjkaša sve je izraženija u zadnjem desetljeću diljem Sredozemlja, što dovodi do znatnih proizvodnih gubitaka i narušava ekonomsku stabilnost sektora (Peteiro i sur., 2010; Šegvić-Bubić i sur., 2011c; Glamuzina i sur., 2014). U istočnom Jadranu, tijekom ljetne sezone kad je metabolizam grabežljivca najaktivniji, tjedni gubitci novonasađenih dagnji iznose više od 50 % nasada (Šegvić-Bubić i sur., 2011c). Pored svojstva uzgajališta da privlači prirodna ihtionaselja (FAD utjecaj) (Morrisey i sur. 2006), zbog nedostatka kompeticije s drugim ribljim vrstama u područjima gdje se odvija uzgoj dagnje i kamenice mnogobrojnim komarčama školjkaši predstavljaju glavni i siguran plijen (Glamuzina i sur., 2014). U Malostonskom zaljevu, najvećem proizvodnom području školjkaša u Hrvatskoj, gubici su u uzgoju cjelogodišnji. Naime, u širem se području zaljeva odvija i uzgoj komarče u kavezima, a mlađ je često podrijetlom iz Sredozemlja ili Atlantika. Epizode zbjega učestale su te se odbjegla riba uspješno integrirala s lokalnim populacijama budući da stanište obiluje lako dostupnim plijenom, školjkašima. Prisustvo prebjega u zaljevu potvrđeno je na morfološkoj i molekularnoj razini te je uočena značajna razina introgresije uzgojnih genotipova uslijed križanja prebjega s autohtonim populacijama (Šegvić-Bubić i sur., 2011a; 2014). Unatoč značajnim socio-ekonomskim i ekološkim posljedicama koje zbjegovi lubina i komarče čine na području Sredozemlja, sustavni pristup proučavanju njihova utjecaja, kao i razvoja preventivnih mjera još nije uvijek uspostavljen (Arechvala-Lopez i sur., 2017; Šegvić-Bubić i sur., 2017).

Svojstva fenotipske plastičnosti u bio-ekološkim istraživanjima

Fenotipska plastičnost predstavlja sposobnost jednog genotipa[10] da u heterogenim okolišnim uvjetima formira alternativne fenotipove (Swain i Foote, 1999). Plastični odgovori poput promjena u ponašanju, fiziologiji, morfologiji, alokaciji bioenergetskih resursa ili dinamici rasta omogućavaju jedinkama da usklade svoje fenotipove s vremenskim i prostornim promjenama biotičkih i abiotičkih čimbenika u danom okruženju (Scheiner, 1993; Nylin i Gotthard, 1998; Schlichting i Pigliucci, 1998). Brojni organizmi posjeduju nasljedno svojstvo plastičnog odgovora na okolišne uvjete svoje životne sredine, što predstavlja važan način održanja fenotipske raznolikosti i značajan pokretač razvoja organizma i njegova funkcioniranja te genetskih promjena populacija vrsta (Scheiner, 1993; Pigliucci, 2001). U nedavnoj teorijskoj sintezi, Pfennig i sur. (2010) istakli su da plastičnost može utjecati na povećanje biološke raznolikosti (i) formiranjem novih fenotipova i divergencijom unutar populacije; (ii) evolucijom divergencije između populacija i vrsta te kroz procese (iii) specijacije i adaptivne radijacije.

Fenotipska plastičnost zauzima važno mjesto u nastanku i održavanju fenotipskih razlika u populacijama te jedinkama služi kao svojevrsna poveznica između starih i novih sredina, pružajući im mogućnost da opstanu u izmijenjenim okolišnim uvjetima i eventualno odgovore prilagodbe na novonastale životne uvjete (West-Eberhard, 2003; Ghalambor i sur., 2007; 2015). Razina ispoljavanja promjene pojedinog obilježja nekog genotipa pod djelovanjem različitih okolišnih uvjeta predstavlja mjeru plastičnosti obilježja koja je uglavnom određena jačinom i brzinom plastičnog odgovora na izmijenjene okolišne uvjete (Schlichting i Pigliucci, 1998). Stoga plastičnost jedne osobine može izmijeniti veći broj međusobno povezanih fenotipskih osobina jedinki (Slika 5), pogotovo ako su izložene različitim izvorima hrane, razinama kompeticije ili rizicima od predacije (Van Buskirk i Steiner, 2009). Primjerice, određena morfološka promjena cjeline često je praćena promjenama u ponašanju ili fiziologiji.

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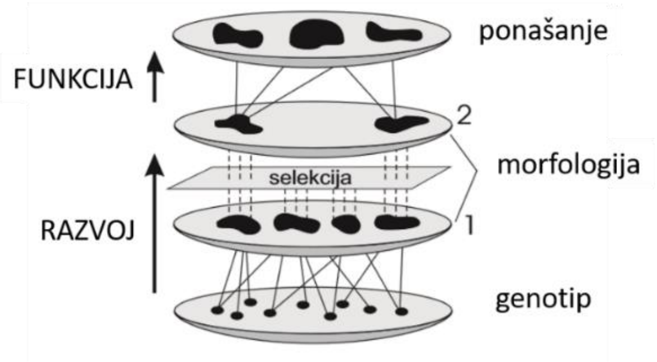
[10] *Genotip* podrazumijeva skup svih gena jednog organizma koji sadrže nasljedna uputstva za formiranje organa i funkcija organizma.

Povećana sposobnost učenja, kao jedno od obilježja fenotipske plastičnosti, omogućava organizmima da što učinkovitije iskoriste okruženje u kojem se nalaze, potičući prilagodbe tjelesnih struktura važnih za npr. iskorištavanje novih trofičkih resursa (Sluys, 1996; Schlichting i Pigliucci, 1998).



Slika 5. Odnosi osnovnih razina biološke organizacije (genotip, morfologija, ponašanje). Plastičnost nije odlika organizma već njegovih pojedinačnih karakteristika na svim razinama fenotipa i u svim fazama životnog ciklusa. Procesi razvoja povezuju razine genotipa i morfologije, a funkcija razinu morfologije i ponašanja (modificirano prema Sluys, 1996).

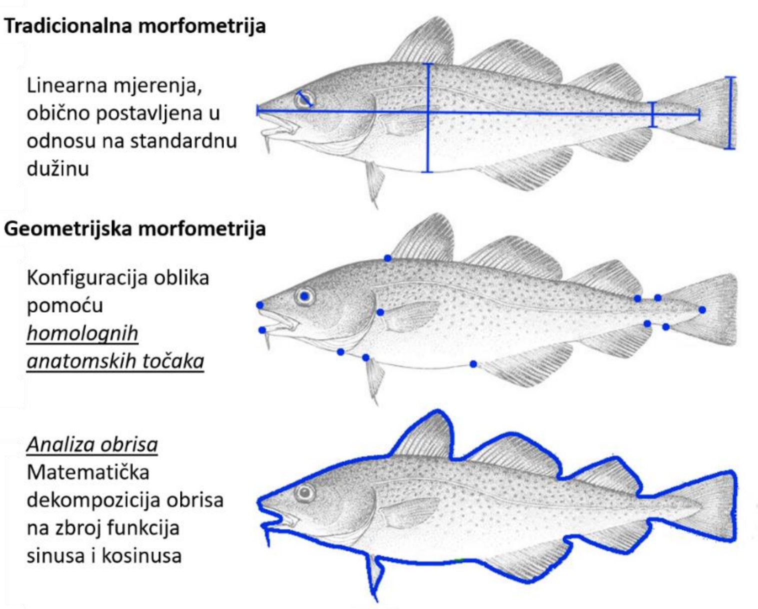
Geometrijska morfometrija u ihtiologiji

Morfometrija predstavlja područje morfologije kojom se opisuju biološke cjeline kvantifikacijom morfološke varijabilnosti, uzimajući u obzir njihovu veličinu i oblik. Kao znanstvena disciplina, doživjela je procvat od 70-ih godina prošloga stoljeća naovamo zbog razvoja elektroničkih računala koja su omogućila primjenu multivarijatnih statističkih metoda na velikom broju varijabli (Zelditch i sur., 2012). U morfološkim studijama uglavnom se koriste tipovi podataka u obliku pravocrtnih dužinskih mjera te konfiguracija specifičnih točaka, dok se u ihtiološkim istraživanjima najčešće primjenjuju klasična metoda tjelesnih odnosa i geometrijska morfometrija (Slika 6), koja uključuje upotrebu homolognih anatomskih točaka (eng. *landmarks*) i polutočaka krivina (eng. *semilandmarks*) te analize obrisa ili kontura (eng. *outline*).

Klasična metoda odnosa u ribarstvenoj biologiji zasniva se na pravocrtnim udaljenostima među točkama, gdje se morfometrijske tjelesne osobine uspoređuju s odgovarajućom tjelesnom veličinom radi dobivanja relativnih odnosa (u %). Stoga su tjelesne osobine mjerene na glavi (predočni prostor, promjer oka, zaočni prostor) izražene u odnosu na dužinu glave, dok su sve ostale tjelesne veličine izražene u odnosu na standardnu dužinu tijela (Treer, 1993).

Konfiguracije specifičnih točaka odgovaraju jasno definiranim morfološkim točkama koje je moguće s velikom preciznošću odrediti kod svih jedinki u analiziranom uzorku. Odabir specifičnih točaka zasniva se na dobrom poznavanju anatomije organizma koji se istražuje, gdje raspored specifičnih točaka treba ocrtavati oblik analizirane tjelesne strukture. Odabir broja specifičnih točaka ovisi o samom objektu istraživanja tj. broju mogućih točaka na samom objektu, od istraživačkog cilja, kao i o veličini dostupnog uzorka (Zelditch i sur., 2012).

Analiza obrisa ili kontura primjenjuje se ako je na ihtiološkim objektima broj jasno definiranih homolognih anatomskih točka, koje se mogu ustvrditi s velikom ponovljivošću, jako mali ili u potpunosti nedostaje (Lishchenko i Jones, 2021). U takvim se slučajevima što veći broj točaka duž vanjskoga ruba (obrisa) proučavanog objekta digitalizira i uklapa u matematičku funkciju, kao npr. Fourierova transformacija, te se uspoređuju uzorkovane krivulje pomoću Fourierovih koeficijenata kroz multivarijatne statističke metode. Točke u multivarijatnom prostoru mogu se transformirati natrag u fizički prostor analiziranog objekta i vizualizirati kao obris uzorkovane strukture (Bonhomme i sur., 2014).



Slika 6. Komparativni pregled morfometrijskih metoda u ihtiološkim istraživanjima (Izvor: Bonhomme, 2018).

Pravocrtne mjere sadrže manje informacija u odnosu na metode geometrijske morfometrije budući da se veliki broj mjera međusobno preklapa ili su usmjerene u sličnome smjeru. Nekolicina mjera proizlazi iz iste točke što uvjetuje izostanak nezavisnosti varijable, dok pogreška u lociranju točke utječe i na vrijednosti više povezanih mjera. Primjerice, tjelesna visina najčešće je definirana pravocrtnom udaljenošću između anteriorne baze leđne peraje do anteriorne baze analne peraje, ali također može biti određena najvećom visinom tijela. Najveća tjelesna visina nije anatomski kompatibilna od vrste do vrste, pa čak i od jedinke do jedinke te posljedično ne pruža dodatnu informaciju za populacijske ili filogenetske analize, osim one o samoj visini tijela jedinke. Stoga je glavni nedostatak tradicionalnog metodološkog pristupa izostanak mjera povezanih s homolognim obilježjima organizama te isključivo numerički prikaz mjerenih vrijednosti bez grafičkog prikaza, što znatno otežava tumačenje rezultata (Zelditch i sur., 2012). Ovaj osnovni koncept i tip podataka u morfometriji koristi se već desetljećima i pripada tradicionalnoj morfometriji (Treer, 1993). Međutim, početkom 90-ih godina prošloga stoljeća došlo je do razvoja velikoga broja novih morfometrijskih metoda koje se mogu svrstati u novu znanstvenu disciplinu nazvanu geometrijska morfometrija. Tim su se metodama nastojali ukloniti nedostaci tradicionalne morfometrije, ali ubrzati i pojednostavniti pridobivanje podataka s bioloških objekata kroz njihovu digitalizaciju (Cardini i Loy, 2013).

Sve je veći istraživački interes u suvremenijoj ihtiologiji usmjeren na ekomorfologiju, podznanstvenu disciplinu koja promatra odnose morfoloških karakteristika ribljih vrsta i njihovih niša[11]. Osnovna premisa ove discipline podrazumijeva da su interspecijske morfološke varijabilnosti u sprezi s položajem vrste u ekološkom prostoru niše (Norton i sur., 1995). Prednost je metoda geometrijske morfometrije što omogućuju izradu grafičkih prikaza proučavanih oblika analizom varijabli, na temelju čega se mogu razmatrati bio-ekološke karakteristike i prilagodbe vrste u promatranim okolišima (Klingenberg, 2013). Vizualno tumačenje rezultata omogućava uočavanje morfoloških specifičnosti na malim geografskim skalama te genetski bliskih ili istih populacija ili taksona riba (Cadrin i sur., 2014).

Konfiguracije specifičnih točaka odgovaraju jasno definiranim morfološkim točkama koje je moguće sa preciznošću odrediti kod svih jedinki u analiziranom uzorku. Podaci oblika multidimenzionalni su odnosno svaki uzorkovani objekt ima konfiguraciju homolognih anatomskih točaka koje su opisane određenim brojevnim koordinatama (X, Y). U matematičkom smislu to omogućava da se oblik promatrane morfološke strukture definira kao zbir geometrijskih informacija koje su nepromjenjive u odnosu na skaliranje, translaciju i rotaciju. Generalna Prokrustova analiza (eng. *General Procrustes Analysis* – GPA) najčešće je primijenjena metoda geometrijske morfometrije čijom se provedbom uklanjaju razlike uvjetovane veličinom, položajem i orijentacijom uzorkovanih jedinki (Slika 7). Prokrustova analiza koristi procese skaliranja, translacije i rotacije konfiguracija homolognih anatomskih

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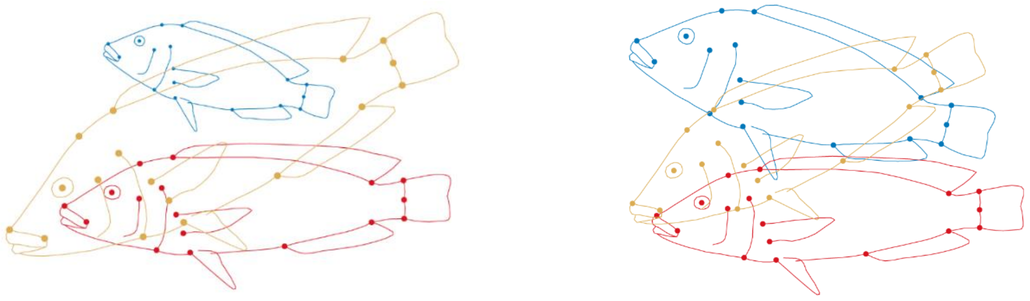
[11] *Niša* je položaj ili status organizma u ekosistemu koji proizlazi iz morfoloških adaptacija, fizioloških reakcija i ponašanja organizama. Ekološka niša nije definirana samo mjestom gdje organizam živi, već i funkcijama koje on obavlja u datom staništu.

točaka na morfološkoj strukturi ribe kako bi zadržala samo informacije koje su vezane za razlike u obliku Prokrustovih koordinata (Klingenberg, 2010).

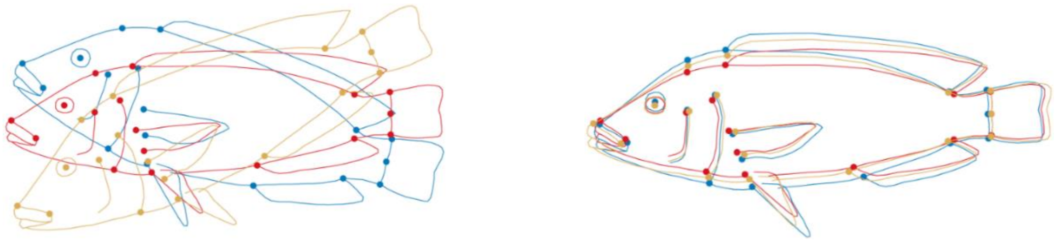
A) Originalni položaj jedinki

B) Skaliranje jedinki na istu veličinu



C) Translacija jedinki prema istom centru tjelesne forme

D) Pozicioniranje jedinki na istu orijentaciju



Slika 7. Proces Prokrustove superimpozicije i izvlačenja geometrijske informacije o morfološkoj konfiguraciji tjelesnog oblika kod riba putem homolognih anatomskih točaka (Izvor: Klingenberg, 2010).

Prokrustove koordinate predstavljaju varijable oblika i polazni su podaci u multivarijantnim statističkim analizama jer pružaju informacije o ukupnoj varijabilnosti oblika koja uključuje alometrijsku (promjene oblika kao rezultat razlika u veličini) i ne-alometrijsku komponentu (promjene oblika kao rezultat djelovanja svih ostalih čimbenika izuzev alometrije). Prokrustova udaljenost, kao linearna mjera udaljenosti između konfiguracija koje se uspoređuju, upotrebljava se u geometrijskoj morfometriji kao osnovna mjera razlika u obliku. Centroidna veličina koristi se kao parametar veličine koja predstavlja mjeru raspršenosti homolognih točaka od središta ili centroida date konfiguracije. Vrijednost centroidne veličine veća je što su homologne točke udaljenije od centra oblika (Zelditch i sur., 2012).

Važna razlikovna karakteristika između analize oblika uzetih konfiguracijom točaka u odnosu na tradicionalne morfometrijske metode jest što je analiza anatomskih homolognih točaka nužno multivarijantna. Informacije o promatranom obliku sadržane su u cijeloj konfiguraciji homolognih anatomskih točaka jer se ona razmatra kao jedinstveni skup podataka. U tradicionalnoj morfometriji svako pojedinačno mjerenje razmatra se kao samostalno obilježje, dok su podaci oblika uzorkovanih konfiguracijom homolognih anatomskih točaka višedimenzionalni. Navedeno omogućava da se svaka pojedina konfiguracija opisana brojnim koordinatama može analizirati u cjelini ili u manje međusobno povezanim ili nezavisnim morfološkim cjelinama (Zelditch i sur., 2012).

Biologija i ekologija komarče *Sparus aurata*

Komarča je riba iz razreda Actinopteri, reda Eupercaria i porodice ljuskavki (Sparidae). Ljuskavke obuhvaćaju veliki broj bentopelagičnih vrsta koje zbog izuzetne kvalitete mesa imaju veliku komercijalnu važnost (Basurco i sur., 2011) te su u Sredozemnom moru prisutne s rodovima: *Dentex* (Cuvier, 1814), *Diplodus* (Rafinesque, 1810), *Pagellus* (Valenciennes, 1830), *Pagrus* (Cuvier, 1816), *Lithognathus* (Swainson, 1839), *Spondyliosoma* (Cantor, 1849), *Oblada* (Cuvier, 1829), *Crenidens* (Valenciennes, 1830), *Boops* (Cuvier, 1814), *Sarpa* (Bonaparte, 1831) i *Sparus* (Linnaeus, 1758). To su srednje do velike ribe veličinskog raspona koji se kreće od par desetaka centimetara do jednog metra dužine, s oblikom tijela koje je više-manje bočno spljošteno s vretenastim ili ovalnim oblikom tijela. Većina pripadnika ove porodice su dvospolci, a hrane se školjkašima, rakovima i ribom, a djelomično i morskim biljem (Jardas, 1996; Moretti i sur., 1999).

Komarču karakterizira izduženo, ovalno i bočno spljošteno tijelo s konveksnim profilom glave, odebljanim usnama te zlatnožutim mostom koji se proteže između malih očiju (Slika 8). Na početku bočne pruge nalazi se veća ljubičastocrna mrlja koja zahvaća i dio škržnog poklopca, na čijem se dijelu nalazi živozlatna do narančasta mrlja. Leđa su modrikasto do zelenkastosivog i kovnoga sjaja, dok su bokovi srebrenkasti s smeđozlatkastim ili smeđim prugama. Donji je dio tijela srebrnastobijel. Po sredini leđne peraje nalazi se crna pruga. Usta imaju mandibulu koja je kraća od maksile te se sprijeda u objema čeljustima nalaze četiri do šest snažnih očnjaka s bočnim zubima u obliku kutnjaka, poredanih u dva do četiri niza. Ktenoidne ljuske postoje na obrazima i škržnom poklopcu, ali ne i na pretpoklopcu. Na bočnoj pruzi nalazi se od 75 do 85 ljusaka. Leđna je peraja dugačka kao i šiljasta prsna peraja dok je trbušna peraja mnogo kraća od prsnih. Repna peraja račvasta je s crnim obrubom (Jardas, 1996; Moretti i sur., 1999).

Slika 8. Jadranska divlja komarča (Izvor fotografije: Igor Talijančić).

U Sredozemnome moru, razdoblje mrijesta komarče odvija se od listopada do prosinca u temperaturnom rasponu od 13 do 17^{o}C. S obzirom na spolnost, komarča je protandrični dvospolac čije su jedinke funkcionalno mužjaci prve dvije godine starosti, obično pri dužinama od 20 do 30 cm i masama od 100 do 300 g. Tijekom treće godine mijenjaju spol u ženku. Asinhroni razvoj jajnika i testisa omogućava višekratno izbacivanje jaja tijekom produžene reproduktivne sezone, koja može potrajati dva do tri mjeseca (Moretti i sur., 1999).

Kao suptropska vrsta, komarča je široko rasprostranjena u istočnom Atlantiku te naseljava područja od sjevernog Britanskog otočja do Kanarskih otoka te zapadnu Afriku, sve do obale Senegala. Kako je komarča toploljubna vrsta, povećanje temperature uslijed klimatskih promjena u posljednjih deset godina pogodovalo je proširenju geografske rasprostranjenosti populacija i prema sjevernoj polutki, gdje je porast brojnosti vrste zabilježen i uzduž obala Francuske i Britanskog otočja (Coscia i sur., 2012; Avignon i sur., 2017). U Jadranu je rasprostranjena uz obalu te obično obitava iznad pjeskovita ili pjeskovito-ljušturna dna mirnih uvala, zaljeva, ušća rijeka i naselja livada morskih cvjetnica (Jadras, 1996). Rani razvojni stadiji nastanjuju pliće vode gdje formiraju plove dok odrasle jedinke obitavaju u obalnim područjima do dubine od 50m, rjeđe zalazeći dublje od 150 m (Moretti i sur., 1999; Dimitriou i sur., 2007; Sola i sur., 2007). Obitava u gotovo svim morskim i bočatim staništima kao što su obalne lagune i riječni estuariji jer kao vrsta podnosi široki raspon vrijednosti temperatura i slanosti. Poslijeličinački stadiji tijekom zimskog razdoblja obavljaju trofičke migracije prema zaštićenim priobalnim područjima u potrazi za dostatnim količinama hrane i umjerenim temperaturnim uvjetima, dok odrasle jedinke migriraju zbog mrijesta prema otvorenom moru u kasnu jesen zbog njihove osjetljivosti na niske temperature, s donjom letalnom granicom od 4^{o}C (Sola i sur., 2007).

Fenotipska obilježja populacija komarče u prirodnim i uzgojnim uvjetima

Ribe, kao živi organizmi, izrazito su prilagodljive novim uvjetima okoliša sa stupnjem plastičnosti koji je specifičan svakoj pojedinoj vrsti (Fiske i sur., 2001). Neke populacije riba ispoljavaju slične prosječne fenotipske vrijednosti, odnosno stabilne su neovisno o okolišnim uvjetima, dok druge pak pokazuju veću fenotipsku plastičnost. Varijabilnost fenotipova u prirodnim ihtiopopulacijama uglavnom ovisi o morfološkom stupnju prilagodbe utjecajima brzine morskih strujanja, rizika od predacije te dostupnim izvorima hrane (Webb, 1984; Walker, 1997; Colihueque i Araneda, 2014). Međutim, osim što je zaslužna za fenotipske razlike između uzgojnih populacija koje su svojstvene vrsti, plastičnost se u zatočeništvu ispoljava i kroz pojedina obilježja koja su zajednička svim uzgojnim ribama, bez obzira na domestifikacijsku razinu. Sve riblje vrste u uzgoju imaju manju glavu i veličinu gornje čeljusti, kao i kraće leđne te repne peraje zbog kontrolirane hidrodinamike uzgojnih jedinica, gustoće nasada te dostupnosti hrane (Wringe i sur., 2016).

Dosadašnja istraživanja. Antropogene aktivnosti na području Sredozemlja predstavljaju sve veću ugrozu za okoliš budući da su rastuće uslužne i proizvodne djelatnosti poput turizma, plovidbe, iskorištavanja energetskih resursa, ribolova i akvakulture značajno intenzivirale utjecaj na morsku biotu od 1990. godine (Malak i sur., 2015). Utjecaji akvakulture na okoliš prvotno su promatrani kroz procjenu pritiska organskih tvari i nutrijenata na bentoske zajednice i vodeni stupac, dok je utjecaj bježanja ribe bio zapostavljen. Međutim, porast uzgojnih kapaciteta morskih vrsta riba tijekom posljednjeg desetljeća popraćen je i velikim brojem zbjegova, unatoč tehnološkim i sigurnosnim poboljšanjima infrastruktura uzgajališta. Jackson i sur. (2015) objavili su da je gotovo devet milijuna riba pobjeglo iz mrežnih kaveza u šest europskih zemalja, što predstavlja ozbiljan rizik za okoliš koji je prouzrokovan uzgojem riba. S obzirom na rastuću zabrinutost o potencijalnim utjecajima zbjegova na autohtone populacije, znanstvena je zajednica uspostavila i provela niz znanstvenih projekata financiranih iz europskih fondova poput:

- GENIMPACT (6th Framework Programme for research FP6) – Evaluation of genetic impact of aquaculture activities on native populations;
- AQUATRACE (7th Framework Programme for research FP7) – The development of tools for tracing and evaluating the genetic impact of fish from aquaculture;
- PREVENT ESCAPE (7th Framework Programme for research FP7) – Assessing the causes and developing measures to prevent escape of fish from sea-cage aquaculture.

U sklopu "Post-escape" istraživačkog paketa projekta "Prevent escape" identificirane su osobine zbjegova komarče i lubina te njihova raspodjela, moguće ekološke posljedice i metode ponovnog ulova. Na osnovi analize troška i dobiti pokazatelja i opisnih karakteristika prirodnih i uzgojnih obilježja komarče i lubina (Tablica 10), Sanchez-Jerez i sur. (2013) predložili su karakteristike vanjskog izgleda, morfometrije i genetike kao one koje imaju najbolju primjenjivost jer brzo i točno daju potrebne informacije koje najbolje pokrivaju interese većine dionika morskog ribarstva. Prosječni tjelesni oblik komarče u uzgoju opisan je s plosnatijim i zaokruženijim profilom glave te zdepastijim tijelom u odnosu na divlje jedinke izduženijeg tjelesnog oblika s manjom tjelesnom visinom i većom glavom (Tablica 11).

Tablica 10. Razlikovni pokazatelji uzgojnih i divljih komarči te učinkovitost njihove primjenjivosti: bijela boja označava najmanju dok crna boja visoku točnost pokazatelja u odnosu na promatranu skupinu. Analiza troška i dobiti prikazuje koji opisni pokazatelji pružaju najviše informacija s najmanjim troškom (Izvor: Sanchez-Jerez i sur., 2013).

	VI			M/S			BK/SMK		SI		ET		GE
Sparus aurata	Boja	Peraje	Ljuske	Oblik	Kondicija	Otoliti	Lipidi	LA, ARA	δ13C	δ15N	Ljuske	Otoliti	DNK
Učinkovitost	░		■	▩	■	░	░	▩	▩				▩
Brzi odgovor		░		■	■	▩	▩	░			▩	■	
Vremenska postojanost									▩	░			■
Gospodarenje	▩	▩	▩	■	▩	░							
Tržište (prodavači, potrošači)	▩	▩	░		■		▩						
Uzgajivači	▩	▩	▩	▩	■	░		░	░	░			
Okolišno upravljanje	▩	▩	▩	■	▩	▩		▩	▩	░	▩	░	▩
Jedinka				▩	░	░	▩	░	░				■
Podrijetlo uzgojnog stoka									░		▩	▩	■

Analiza troška i dobiti primjene opisnih pokazatelja između uzgojnih i divljih jedinki			
Komarča, *S.aurata*		Lubin, *D. labrax*	
Indeks kondicije	1	Indeks kondicije	1
Morfometrija	0,96	Morfometrija	0,93
Obilježja ljusaka	0,96	Profil masnih kiselina	0,88
Vanjski izgled	0,92	Elementi u tragovima kod ljusaka	0,80
Profil masnih kiselina	0,89	Genetske metode	0,78
Genetske metode	0,86	Obilježja ljusaka	0,64

* VI – vanjski izgled; M/S – morfometrija i somatometrija; BK – biokemijska kompozicija; SMK – Sadržaj masnih kiselina (LA – linoleinska kiselina, ARA – arahidonska kiselina); SI – stabilni izotopi; ET – elementi u tragovima; GE – genetika.

Tablica 11. Karakteristična obilježja komarče divljeg i uzgojnog podrijetla (Izvor: Arechavala-Lopez i sur., 2013).

Podrijetlo	Uzgojno	Divlje
Oblik tijela	Viša tjelesna visina i manja trupna regija Visoki indeks kondicije Visoki relativni profilni indeks	Mala tjelesna visina Mali indeks kondicije Mali relativni profilni indeks
Zubi	Mali i slabo razvijeni Zaobljeni i kvadratastog oblika	Jako razvijeni Oštri i koničnog oblika
Koža	Tamna obojenost bez sjaja, pogotovo na području glave i leđa	Tijelo svijetle i iridescentne boje
Ljuske	Tvrda koža s oskudnom pokrivenošću ljuskama Regenerirani nukleus	Tanja koža sa gustom pokrivenošću ljuskama Čisto vidljiv nukleus
Peraje	Visoki stupanj erozije repne i prsne peraje	Neznatna ili nikakva perajna erozija

Razlike u tjelesnim obilježjima divljih i uzgojnih populacija komarči (Tablica 12) istraživane su tradicionalnim morfometrijskim pristupom, metodom vezane mreže (Arechavala-Lopez i sur., 2012a; Rogdakis i sur., 2011; Šegvić-Bubić i sur., 2014) te klasičnom metodom tjelesnih odnosa (Grigorakis i sur., 2002; Šegvić-Bubić i sur., 2014), dok je geometrijska morfometrija primijenjena u manjem broju studija (Loy i sur., 1999; Coban i sur., 2008; Fragkoulis i sur., 2016). Raznolikost fenotipova unutar uzgojnih populacija povezuje se s geografskim podrijetlom jedinki te s različitim proizvodnim uvjetima gdje gustoća nasada, strategija hranjena i selekcijski ciljevi proizvođača imaju najveći utjecaj na pojavnost robusnosti i zbijenosti tijela kod komarči i lubina (Arechavala-Lopez i sur., 2012a; Grigorakis i sur., 2002). Također, nekolicina je autora zabilježila i da intenzivnija ishrana tijekom toplijeg dijela godine doprinosi nakupljanju lipida u tkivima trbušne šupljine, što još više naglašava zdepasti oblik uzgojnih populacija (Grigorakis i sur., 2002; Hurtado-Rodriguez i sur., 2010; Popović i sur., 2012).

Osim zapaženih razlika na razini tjelesnog oblika, uočena je i značajna varijabilnost obojenosti kože s obzirom na podrijetlo komarče. Pavlidis i sur. (2011) uočili su da vrsta i kvaliteta hrane imaju značajan utjecaj na fenotipska obilježja vrsta iz porodice ljuskavki jer u velikoj mjeri određuju boju i izgled ribe. Riblja hrana s ograničenim udjelom karotenoida te visoka gustoća riba u kavezima glavni su čimbenici odgovorni za depigmentaciju uzgojne komarče i ispoljavanje tamnije i blijeđe obojenosti tijela, bez karakterističnog srebrnkasto-sivkastog sjaja i iridescetnih boja koje se opažaju kod divljih jedinki (Grigorakis i sur., 2002; Pavlidis i sur., 2011; Arechavala-Lopez i sur., 2013).

Tablica 12. Dosadašnja istraživanja tjelesnih karakteristika divljih i uzgojnih komarči u Sredozemnome moru.

Lokacija	Morfometrijska metoda	Oblik tijela	Peraje	Kondicija	Autori
Španjolska	Metoda vezanih mreža[12]	Divlje i uzgojne jedinke pokazuju slične dužine glave, iako su divlje komarče okarakterizirane sa šiljatijom gubicom; Divlje jedinke imaju manju tjelesnu visinu i vretenastije tijelo.		Uzgojna riba ima veću kondiciju u odnosu na divlje jedinke	Arechavala-Lopez i sur. (2012)
Grčka	Klasična metoda odnosa Metoda vezanih mreža Geometrijska morfomatrija	Uzgojne komarče imaju dužu glavu u usporedbi s divljim i poribljavanim jedinkama; Divlje komarče okarakterizirane su s manje naglašenim abdominalnim dijelom tijela, vitkijim trupom, manjom glavom i kraćim repnim drškom; Uzgojne jedinke imaju glomaznije i zdepastije tijelo s većom tjelesnom visinom u odnosu na izduženije tijelo divljih i poribljavanih jedinki.	Uzgojne ribe imaju kraće peraje u odnosu na divlje i poribljavane jedinke.	Uzgojna riba ima veću kondiciju u odnosu na divlje jedinke.	Grigorakis i sur. (2002); Rogdakis i sur. (2011); Arechavala-Lopez i sur. (2012); Fragkoulis i sur. (2016)
Francuska	Geometrijska morfometrija	Uzgojne jedinke pokazuju zaokruživanje cijele frontalne regije gdje profil glave ne sliči uopće tipičnom divljem; Divlje komarče imaju vitkije tijelo i aerodinamičniju anteriorno-frontalnu regiju.	Francuske uzgojne jedinke imaju skraćeniju dužinu analne peraje u usporedbi s jedinkama španjolskoga podrijetla		Loy i sur. (1999)
Turska	Geometrijska morfometrija	Divlje jedinke imaju vitkije tijelo s manjom tjelesnom visinom u odnosu na uzgojne.			Coban i sur. (2008)
Hrvatska	Klasična metoda odnosa Metoda vezanih mreža	Divlje i uzgojne komarče malostonskog zaljeva imaju slične dužine glave, iako su uzgojne jedinke okarakterizirane s plosnatijim profilom u odnosu na divlje jedinke. Uzgojne komarče imaju zdepastije tijelo dok je tjelesni oblik divljih jedinkin više izdužen, s manjom tjelesnom visinom.	Divlje jedinke imaju dužu prsnu, analnu i repnu peraju te veću leđnu peraju	Uzgojna riba ima veću kondiciju u odnosu na divlje jedinke.	Šegvić-Bubić i sur. (2014)

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[12] Metoda vezane mreže (engl. *truss network*) pripada tradicionalnoj morfometriji putem koje se tjelesni oblik uzorkuje dužinskim mjerenjima između homolognih anatomskih točaka.

Uz vanjska tjelesna obilježja, ljuske i otoliti također su korišteni kao fenotipski biljezi za razlikovanje divljeg od uzgojnog podrijetla komarče budući da se primjenjuju u ihtiološkim istraživanjima pri identifikaciji ribljih stokova, određivanju dobi ili veličine ribe (Arechevala-Lopez i sur., 2013). U odnosu na ljuske divljih jedinki koje imaju oblik mnogokuta, najviše peterokuta ili oblika čaške sa širokim anteriornim područjem i konveksnim bočnim stranama, uzgojne su bočno izduženije s regeneriranim nukleusom[13] te većim broj radija[14] (Arechavala-Lopez i sur., 2012c; Šegvić-Bubić i sur., 2020). U zatočeništvu komarče obitavaju u uvjetima visoke gustoće nasada i učestalih zootehničkih manipulacija koje uključuju učestaliji fizički kontakt i trenje. Regenerirana ljuska u središtu nema koncentrične krugove, zone prirasta i nukleus što pogoduje njenoj upotrebi pri identifikaciji zbjegova (Arechavala-Lopez i sur., 2012c). Oblik otolita divljeg podrijetla izduženi je i nepravilan peterokut s izduženim i širokim rostrumom (Tuset i sur., 2008; Arechavala-Lopez i sur., 2012c), dok je uzgojni karakteriziran s urezom između vršnih dijelova na anteriornom dijelu otolita (Geladakis i sur., 2020). Kod divljih komarči oblik otolita je podložan promjenjivim okolišnih čimbenicima te razdobljima gladovanja u odnosu na uzgojne jedinke koje zbog stalnog priljeva riblje hrane drugačijeg biokemijskog sastava imaju brži rast, veće otolite i masu (Arechavala-Lopez i sur., 2012c; Geladakis i sur., 2020).

Osim morfoloških obilježja tijela, ljuski i otolita, odnos postotnog udjela ukupne masti i sadržaja vode u kemijskom sastavu mesa pokazao se korisnim za prepoznavanje zbjegova unutar prirodnih ihtiopopulacija (Popović i sur., 2012; Arechavala-Lopez i sur., 2013). U zatočeništvu se ribe hrane ekstrudiranim peletima koji imaju povećani sadržaj masti u odnosu na sadržaj bjelančevina, što pridonosi nakupljanju masti u tkivima uzgojnih populacija u iznosu i do 20 %, dok se prosječne vrijednosti kod divljih riba kreću oko 1,5% (Grigorakis, 2007; Arechavala-Lopez i sur., 2013; Tičina i sur., 2020). Fernandez-Jover i sur. (2011) pokazali su da ishrana ribe hranom antropogenog podrijetla utječe i na profil masnih kiselina budući da se u proizvodnji riblje hrane sve više uklapaju sastavnice kopnenog podrijetla, poput biljnih ulja koja sadrže visoke koncentracije oleinskih, linolnih i α-linolenskih masnih kiselina. Kod uzgojnih populacija komarči najčešće je zabilježen visoki udjel linolne kiseline u tkivu koja se nakuplja uglavnom nepromijenjena u lipide morskih riba, uslijed slabijeg kapaciteta za elongaciju i desaturaciju (Arechavala-Lopez i sur., 2013).

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[13] Nukleus je prvi dio koji nastaje tijekom ontogenetskog razvoja ljuske. Obično se nalazi na sredini, iako može biti smješten više prema anteriornom ili posteriornom području ljuske.

[14] Radiiji su brazde na ljuskama koje se šire od nukleusa prema vanjskom rubu.

CILJEVI I HIPOTEZE ISTRAŽIVANJA

Fenotipska obilježja populacija komarči u istočnom dijelu Jadranskoga mora još uvijek nisu dovoljno istražena. Dosadašnja znanstvena istraživanja većinom su bila usmjerena na utvrđivanje genetske raznolikosti i strukture prirodnih populacija kao i na utjecaj akvakulture na izvornost prirodnih populacija kroz križanje zbjegova s lokalno-divljim populacijama (Šegvić-Bubić i sur., 2011a; 2014; Žužul i sur., 2019). Uzimajući u obzir trend razvoja akvakulture, navike potrošača kao i sve veću brojnost komarče u prirodnim staništima, ukazuje se potreba za sveobuhvatnim istraživanjem fenotipskih obilježja komarče na području istočnog Jadrana.

Predmetno istraživanje provedeno je kako bi se stekla nova saznanja o utjecaju zbjegova iz akvakulture na prirodne populacije komarče. Budući da ribe izbjegle iz akvakulture predstavljaju ekološku i socio-ekonomsku prijetnju okolišu i potrošačima, neophodno je razvijanje novih pristupa u njihovu prepoznavanju što će unaprijediti upravljanje akvakulturom kao održivom gospodarskom granom izradom nacionalne strategije upravljanja zbjegovima. Također, nužno je provesti vrednovanje tunogojilišta kroz utjecaj na lokalne populacije kako bi se dodatno rasvijetlila njihova potencijalna uloga kao lokaliteta poistovjećenih sa zaštićenim morskim područjima, gdje odljev ribe može pozitivno utjecati na lokalno ribarstvo i zajednice morskih organizama.

Kako bi se utvrdila raznolikost fenotipskih obilježja, u predmetnoj doktorskoj disertaciji istraživane su populacije komarči iz sjevernog, srednjeg i južnog Jadrana. U istraživanju su uključene i uzgojne populacije s različitih lokaliteta duž jadranske obale jer se većina hrvatskih uzgajališta snadbijeva ranim razvojnim stadijima iz inozemnih mrijestilišta, čiji su fenotipovi uglavnom uvjetovani geografskom specifičnošću matica, proizvodnjom u različitim uvjetima uzgojne sredine te zasebnim selekcijskim ciljevima proizvođača na željena obilježja.

U provođenju mjera očuvanja raspoznavanje prebjegle ribe iz akvakulture predstavlja prvi korak. Iako su se molekularne metode pokazale kao jako učinkovit alat pri identifikaciji zbjegova komarče i lubina (Šegvić-Bubić i sur., 2011a; 2017; Žužul i sur., 2019), njihova primjena iziskuje specifičnu znanstvenu i tehnološku podršku te veće financijske izdatke jer prosječna cijena identifikacije podrijetla po pojedinom uzorku iznosi oko 65 USD (Martinsohn i sur., 2018). Stoga bi razvoj brzog, jeftinog, a ujedno i preciznog alata temeljenog na morfološkim karakteristikama omogućio bolju kontrolu uvida u podrijetlo lovine. Međutim, upotreba metoda geometrijske morfometrije pri identifikaciji podrijetla jedinki predstavlja i znanstveni izazov budući da čimbenici poput prirodne ishrane, smanjene populacijske gustoće kao i prirodna selekcija mogu utjecati na uspješnost prepoznavanja zbjegova jednom kad se nađu u prirodnim uvjetima, gdje se uzgojno karakteristični fenotip može potpuno izgubiti odnosno poprimiti divlji.

Ova doktorska disertacija uključuje tri samostalna znanstvena članka (I – III) koja su na odgovarajući način obradila ciljeve i hipoteze. Ciljevi predmetne doktorske disertacije jesu:

- istražiti bio-ekološka obilježja populacija komarči u istočnom dijelu Jadranskoga mora, kako bi se razvio brz i precizan alat raspoznavanja divljih i uzgojnih jedinki koristeći se metodama geometrijske morfometrije te;
- procijeniti utjecaj tunogojilišta na fenotipska svojstva populacija komarči koje obitavaju u okolici kaveznih infrastruktura.

Hipoteze ovog istraživanja jesu:

1. Kavezno povezane komarče stalni su rezidenti uzgajališta tuna koji se hrane ostacima male plave ribe (Članak I. i III.);
2. Kondicijski status te reproduktivni potencijal i obilježja komarče određeni su uvjetima staništa i sastavom hrane (Članak I., II., III.);
3. Obojenost, oblik tijela i otolita divljih, kavezno povezanih i uzgojnih komarči razlikuju se (Članak II. i III.);
4. Metode geometrijske morfometrije raspoznaju podrijetlo komarče na temelju tjelesnog oblika i obrisa otolita (Članak II. i III.).

SAMOSTALNI ZNANSTVENI ČLANCI

I. ČLANAK

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Ecological role of bluefin tuna (*Thunnus thynnus*) fish farms for associated wild fish assemblages in the Mediterranean Sea

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ABSTRACT

The ecological effects of tuna fish farms are largely undocumented. This study confirmed their high capacity to attract surrounding wild fish. The aggregation effect persisted year round, without detectable seasonal differences. Farm impact was restricted to close proximity of the sea cages, and was more prominent over the bottom than in the water column strata. Tuna fish farms proved to be high energy trophic resources, as indicated by the enhanced fitness status of two focal species, bogue and seabream. Under abundant food supply, seabream appear to allocate the majority of energy reserves to gonad development. Farm associated bogue had greater parasite loads, with no detrimental effect on fitness status. Overall, tuna fish farms can be regarded as population sources for aggregated wild fish, and under the no fishing conditions within the leasehold areas, can serve as functional marine protected areas.

1. Introduction

The capture bluefin tuna *Thunnus thynnus* fattening or farming industry as a capture-based practice was introduced in Mediterranean in early 1990s, and its rapid expansion was the direct result of the interest and development of the Japanese sushi and sashimi market (Cardia and Lovatelli, 2007; Ottolenghi, 2008). Due to strong fishing pressure and the need for live tuna, the International Commission for the Conservation of Atlantic Tuna (ICCAT) has been established, providing catch quotas for the Atlantic tuna stock annually. Still, tuna production requires a comprehensive approach to ensure its sustainability given that (i) tuna needs large amounts of highly caloric fresh feed (usually herrings and sardine) that increase fisheries impact and operational costs (ii) tuna are difficult to breed in captivity and production still depends on wild stocks.

Despite its importance, limited information is available on the specific impacts this type of farming has on the surrounding environment and biota (Bacher et al., 2012; Šegvić Bubić et al., 2011). The range of effects documented for aquaculture facilities and operation originate predominantly from studies conducted at inshore, coastal finfish farms, as opposed to semi-offshore locations where tuna farming is located. Such a disproportion in the literature regarding coastal vs. semi-offshore farms is certainly due in part to the differences in their accessibility. There are generally far fewer tuna fish farms, and Croatia provides a good illustration of this, with a current total of 10 leasehold areas registered for tuna farming. On the other hand, coastal farms, cultivating mainly European seabass (*Dicentrarchus labrax*) and Gilthead seabream (*Sparus aurata*), are numerous and spread along the entire coastline of the country (FAO, 2016). Additionally, the logistics of farms operations itself, as well as of conducting any research, become more intricate and demanding at semi-offshore tuna farms. Although insights from coastal farms are informative and can be used as an indication of what to expect at semi-offshore farms, there are also major differences between farmed and associated species, feed type and environment, therefore precluding their direct application to tuna farming, and warranting direct assessments.

One of the widely acknowledged effects of aquaculture installations is the capacity to attract wild fish species. The attraction of large fish aggregations typically occurs at a wide variety of natural and artificial structures in the sea (Dempster and Taquet, 2004), and therefore, fish farms can be viewed as large fish attraction devices (FADs) with constant additional food supply, which likely further enhances the attraction effect compared to traditional FADs (Dempster et al., 2002; Tuya et al., 2005, 2006; Uglem et al., 2014). Strong aggregation response of wild fish to aquaculture installations has for the most part been detected at coastal finfish farms, albeit from different regions of the world and cultivating different fish species (seabass/seabream farms in the Mediterranean: Arechavala-Lopez et al., 2015a, Ballester-Moltó et al., 2015, Dempster et al., 2005, Valle et al., 2007; Canary Islands: Riera et al., 2014; Tuya et al., 2005; Red Sea: Ozgul and Angel, 2013, salmon

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farms in Norway: Dempster et al., 2009, Otterå and Skilbrei, 2014, Uglem et al., 2009; yellowtail kingfish in Australia: Dempster et al., 2004, Tanner and Williams, 2015). Close association with farm cages can affect aggregated fish in a number of ways, e.g. changing their diet, altering body condition and reproductive output, parasite loads, susceptibility to fishing pressure, modifying distribution patterns as well as local assemblage composition and residence times of fish in a given aquaculture concession area (Arechavala-Lopez et al., 2015b; Ballester-Moltó et al., 2015; Boyra et al., 2004; Dempster et al., 2011; Fernandez-Jover et al., 2007, 2008, 2011; Goodbrand et al., 2013). Besides two previous studies focusing on the composition and spatio-temporal dynamics of wild fish aggregations (Bacher et al., 2012; Šegvić Bubić et al., 2011), to date, none of the other aforementioned ecological interactions between fish farming and associated wild fish have been investigated at tuna rearing facilities.

This study took a comprehensive approach to investigating the ecological role of tuna fish farms for wild fish assemblages, aiming to acknowledge whether tuna fish farms generate positive effects for aggregated fish and could thereby be regarded as population sources, or whether they act as ecological traps or population sinks if the impacts proved to be negative (Robertson and Hutto, 2006; Dempster et al., 2011). In this context, the specific objectives of the study were: (i) to investigate the attraction of wild fish to tuna farms at two semi-offshore aquaculture sites in the central Adriatic Sea using a spatio-temporal and depth related sampling design, and (ii) to analyse proxies for fitness, i.e. indices of condition and parasite loads of two common farm-attracted wild fish species, seabream, *Sparus aurata*, and bogue, *Boops boops*, with respect to impact level and season, to reveal possible consequences of altered habitats.

2. Material and methods

2.1. Study area

The study was conducted from October 2015 to September 2016 at the two largest tuna fish farms off the eastern coast of the Adriatic Sea (Fig. 1). Both farms are typical semi-offshore capture-based aquaculture facilities. One is located off the southwest coast of the island Brač, and the second is situated off southeast coast of the island Ugljan. Farming at the Brač farm (BF) was initiated in 2001, with an annual production capacity of 900 t. The Ugljan farm (UF) has been in operation since 2005, with a 1400 t annual rearing capacity. At each farm, tunas are farmed in 6 (BF) and 12 (UF) floating, circular cages with a diameter of 50 m and net depths of 20–25 m, positioned at a distance of 100–150 m from one another. During the study period, the biomass in the BF cages ranged from 800 t (October 2015) to 540 t (August 2016), while cages of UF stocked from 1700 t (October 2015) to 1200 t (August 2016) of bluefin tuna. The cages of both farms are set up over a seabed with similar characteristics (gently sloping, soft, mobile, sandy to muddy bottom) and depth (50–60 m). However, as UF is somewhat further offshore than BF (200 m from shoreline vs. 100–150 m), the sea bottom beneath the cages is exclusively of the sediment type, whereas at BF the sandy seabed is dotted with occasional patches of more rugged bottom with rocky outcrops.

2.2. Sampling design

Fish assemblages were investigated at the BF and UF farm locations, representing farm impact level (F), and at corresponding, nearby control locations of otherwise similar environmental conditions (in terms of slope, depth, exposure, type of substrate and distance from shoreline). Control locations were located at a distance gradient from the farm cages – mid-control (MC) at distances of cca. 500 m and control (C) cca. 1 km away from the farms. At each of the locations, and under all three impact conditions (F, MC and C) two sampling sites were also randomly chosen in order to increase spatial replication. Temporal replications included four sampling campaigns throughout the study – at two random times during the cold season (CS, November–April) and likewise during the warm season (WS, May–August). On each sampling occasion and at each station, survey was performed at four distinct depth strata: surface (3 m), midwater (12 m), cage net depth (25 m) and bottom (50–60 m). Six independent observations of fish assemblages (as replicates) were made in each of the depth strata, resulting in a total of 1152 fish counts. Therefore, the sampling design incorporated six factors to quantify the spatio-temporal and depth related differences in species richness, abundance, composition and structure of fish assemblages associated with tuna fish farms and nearby control locations: Impact (Im; fixed factor with 3 levels, orthogonal); Location (Lo; random factor with 2 levels, orthogonal); Site (Si; random factor with 2 levels, nested within Lo); Depth (D; fixed factor with 4 levels, orthogonal); Season (Se; fixed factor with 2 levels, orthogonal); Time (Ti; random factor with 2 levels, nested within Se).

To evaluate the effects of tuna fish farms on the fitness status of associated wild fish, we focused on two species – bogue, *Boops boops*,

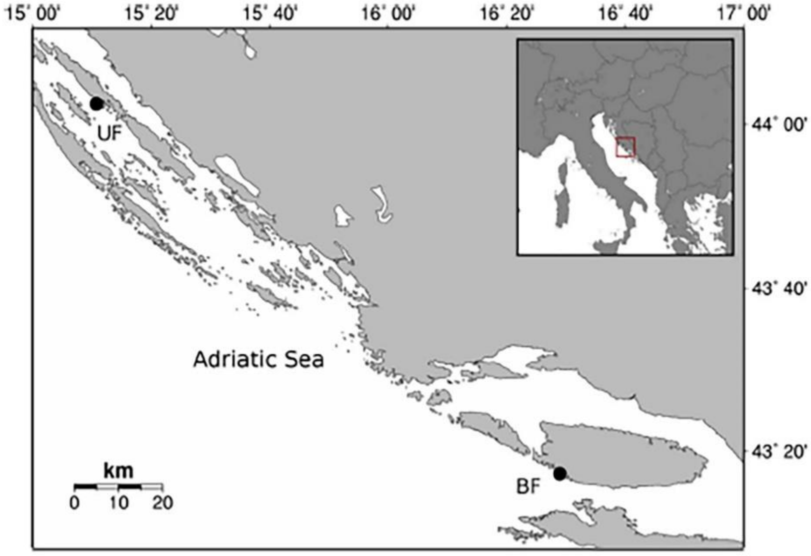

Fig. 1. Investigated tuna-farm locations in the eastern Adriatic Sea. UF, farm off the island of Ugljan; BF, farm off the island of Brač.

and gilthead seabream, *Sparus aurata*. From a previous study (Šegvić Bubić et al., 2011) and personal observations, we were confident that bogue and seabream are abundant enough around fish farms to be caught in sufficient numbers. Additionally, as both species are of commercial value, obtaining samples from non-aquaculture locations was simplified. Farm associated bogue and seabream were obtained from BF and UF locations. Individuals from natural, wild populations were sampled from 6 control locations, each more than 20 km from any aquaculture facilities, to ensure that fish were not in regular interaction with farms. As a proxy for the overall fitness status of bogue and seabream, the following indices were used: relative body condition index (Kn), hepatosomatic index (HI), gonadosomatic index (GI) and parasite loads. Collections of two focal species lasted throughout the year of study. Thus, the design for analysis of differences in fitness status of farm associated and wild fish incorporated two factors: Impact (Im, fixed, 2 levels: 2 farms vs. 6 control locations) and Season (Se, fixed and orthogonal to Impact with 4 levels: spring, summer, autumn, winter).

2.3. Sampling methods

Fish assemblages were studied in line with the above described six-factor sampling design using a stationary stereo-video system AQ1 AM100 (AQ1 Systems, 2016). The system comprised two cameras mounted 0.9 m apart on a neutral buoyancy base bar in waterproof housings. The system was cable-connected to a laptop with software for recording and for subsequent sizing and counting objects.

As water visibility varied at different depths and at different survey times, to ensure good accuracy in fish species identification, the assessment was limited to a maximum distance of 10 m from the camera. With a restricted radius of 10 m, each recording covered a volume of approximately 1.090 m^3 (SCV Calculator, www.aquapop.hr). At each survey site and depth, videos were recorded for a minimum of 15 min. Footage was analysed using the AM100 software that enabled fish distance calculation, slow view, zooming and adjustments to optimize video clarity. Reading of recordings was initiated at least 30 s after the camera reached desired depth in order to minimize the influence of disturbance and camera presence on fish behaviour. On each sampling occasion and for each site and depth, sampled video recordings were analysed for variables of fish species richness (SR) and abundance (SA). SR was measured as the total number of species identified per 1 min of viewing interval. Each minute of recording was considered as one of six replicas in the sampling design, with 30 s of intermittent non-viewing intervals to ensure independency. As counting all the individuals appearing in 1-min replica is impractical, time-consuming and prone to counting the same individuals multiple times, SA was indexed for each replica as the mean count, i.e. as the mean number of fish observed in a series of four snapshots taken over a 1-min viewing interval. This approach has been shown by Conn (2011) and Schobernd et al. (2014) to be a useful metric for enumerating fish in underwater video surveys, as it is generally linearly related to true abundance and with minimal loss of precision.

For the fitness status assessment, a total of 704 bogue and 620 seabream specimens were examined. Farm-associated specimens of target species (422 bogues; 244 seabreams) were captured by hook and line fishing from immediately beneath the cages of the two fish farms studied, while wild fish (282 bogues; 376 seabreams) from control locations not impacted by tuna fish farming were obtained in collaboration with local fishermen or from local fish markets when the catch locality could be verified. All fish were iced upon landing and taken to the laboratory, where they were measured (total length (TL; cm)) and weighed (total weight (TW; g)), then dissected to obtain liver and gonad weights. Sex ratio for bogue (65% female, 35% male) and seabream (25% female, 75% male) was uniform between farm and wild caught specimens. Each specimen was also macroscopically inspected for the presence of external and internal parasites. Three indices were used to provide an indication of fish energy reserves – relative condition factor (Kn), hepatosomatic (HI) and gonadosomatic index (GI), and were calculated as follows: $Kn = TW/TW_r$, where TW is the observed mass of the individual at a given length (TL), and TW_r is the mean weight at that same length (TL) derived from the length-weight relationships ($TW_r = aTL^b$) for the population under study. As comparing the weight of fish against a standard calculated weight avoids the assumption of isometric growth and eliminates potential length effects, Kn was used in preference to Fulton's condition factor ($K = (TW/TL^3)$ x100) (Le Cren, 1951; Lloret et al., 2014); $HSI = (LW/TW)x100$, where LW is liver weight and $GSI = (GW/TW)x100$, GW denoting gonad weight.

2.4. Data analysis

All analyses were conducted with PRIMER version 6 (Clarke and Gorley, 2006) and the PERMANOVA + add-on package (Anderson et al., 2008). Raw data, univariate and multivariate, were treated with square root transformation prior to analysis in order to stabilize the variance and balance between the contributions from common and rarer species, respectively. Prior to transformation, dispersion weighting was also applied to multivariate data to stabilize the differences in variance structure associated with erratic counts of highly spatially clustered species (schooling fish) (Clarke et al., 2006). The null hypothesis of no differences in total fish abundance and number of species observed at each impact level, depth and season of sampling was tested using a six-way univariate PERMANOVA, based on similarity matrixes computed using Euclidean distances. According to the same six-factor sampling design, multivariate PERMANOVA was applied to the Bray–Curtis resemblances of fish assemblage composition and abundance structure data. Statistical significance was set at $p = 0.05$ and p values were obtained using 9999 permutations of residuals under the reduced model (Anderson et al., 2008). Significant terms of the main PERMANOVA tests were further examined by applying appropriate post-hoc pairwise comparisons. Multivariate patterns of fish assemblage composition and structure were visualized using canonical analysis of principal coordinates (CAP, Anderson and Robinson, 2003; Anderson and Willis, 2003) ordinations of centroids of each site. Fish species contributing most to any observed differences highlighted by PERMANOVA were identified by examining Pearson correlations of species counts with canonical axes of the CAP plot. Relationships between individual fish species and the canonical axes with a correlation coefficient (r) $>$ 0.30 are displayed on the CAP ordination as a vector overlay.

Differences in fitness status of farm associated and wild fish for two focal species (bogue, seabream) with respect to impact level and season were tested for each of the indices (Kn, HSI, GSI, parasite loads) using two-way univariate PERMANOVA based on Euclidian distances. To control for any variation in fitness status due to fish size, fish length (TL) was considered as a covariate with the aforementioned factors in a single model. Post-hoc pairwise tests were applied when significant differences were found.

3. Results

3.1. General description of fish assemblages

A total of 12,769 fishes comprising 24 taxa (19 species and 5 genera) and 14 families were recorded throughout the study at both tuna fish farms and the adjacent control locations (Table 1). *Mullus* sp., *Raja* sp., *Spicara* sp., *Trachinus* sp. and *Trachurus* sp. were identified only to the genus level, due to the difficulty of observing species-specific characteristics from video recordings. Comparable values of overall species richness were yielded by both SR and SA viewing procedures. The only species not observed in snapshots was adult greater amberjack, *Seriola dumerili*, that passed by on a single occasion

Table 1
Abundance (mean ± SE) of aggregated, wild fish taxa by depth strata (3 m, 12 m, 25 m, bottom) at each of the impact levels (F = farm; MC = mid-control; C = control).

Family	3 m			12 m			25 m			bottom		
Species	F	MC	C	F	MC	C	F	MC	C	F	MC	C
Belonidae												
Belone belone	0	0	0	0	0	0	0.12 ± 0.04	0	0	0	0	0
Carangidae												
Seriola dumerili	0	0	0	0	0	0	0	0	0	0	0	0
Trachurus sp.	0.10 ± 0.02	0	0	0.24 ± 0.15	0	0	0	0	0	0	0	0
Congridae												
Conger conger	0	0	0	0	0	0	0	0	0	0.02 ± 0.01	0	0
Gobiidae												
Gobius vittatus	0	0	0.01 ± 0.00	0	0	0	0	0	0	0	0	0
Labridae												
Coris julis	0	0	0	0	0	0	0	0	0	0.07 ± 0.02	0.06 ± 0.01	0.02 ± 0.01
Mullidae												
Mullus sp.	0	0	0	0	0	0	0	0	0	0.01 ± 0.01	0.03 ± 0.01	0
Pomacentridae												
Chromis chromis	0	0	0	0	0	0	0	0	0	0.02 ± 0.01	0.03 ± 0.01	0.22 ± 0.06
Rajidae												
Raja sp.	0	0	0	0	0	0	0	0	0	0.01 ± 0.00	0	0
Scorpaenidae												
Scorpaena porcus	0	0	0	0	0	0	0	0	0	0.11 ± 0.02	0	0
Serranidae												
Serranus cabrilla	0	0	0	0	0	0	0	0	0	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Serranus hepatus	0	0	0	0	0	0	0	0	0	0.07 ± 0.01	0.38 ± 0.04	0.01 ± 0.01
Sparidae												
Boops boops	9.95 ± 1.20	0.23 ± 0.07	0.48 ± 0.16	8.67 ± 1.27	0.14 ± 0.05	0.78 ± 0.27	0.25 ± 0.09	0	0	4.67 ± 0.88	0.21 ± 0.13	0.28 ± 0.11
Diplodus annularis	0	0	0	0	0	0	0	0	0	0.15 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Diplodus vulgaris	0	0	0	0	0	0	0	0	0	1.77 ± 0.27	0.52 ± 0.09	0.01 ± 0.01
Oblada melanura	0.01 ± 0.00	0	0	0	0	0	0	0	0	0	0	0
Pagellus acarne	0	0	0	0	0	0	0	0	0	1.27 ± 0.20	0.14 ± 0.05	0.05 ± 0.03
Pagellus erythrinus	0	0	0	0	0	0	0	0	0	0.03 ± 0.01	0.05 ± 0.01	0
Sparus aurata	0	0	0	0	0	0	0	0	0	0.57 ± 0.08	0.09 ± 0.02	0.01 ± 0.00
Spicara sp.	0	0	0.17 ± 0.06	0	0	0.02 ± 0.01	0	0	0	0.51 ± 0.08	0.41 ± 0.07	0.15 ± 0.04
Spondyliosoma cantharus	0	0	0	0	0	0	0	0	0	0	0	0.003 ± 0.0
Torpedinidae												
Torpedo torpedo	0	0	0	0	0	0	0	0	0	0	0.02 ± 0.01	0
Trachinidae												
Trachinus sp.	0	0	0	0	0	0	0	0	0	0.003 ± 0.0	0.06 ± 0.01	0
Zeidae												
Zeus faber	0	0	0	0	0	0	0	0	0	0	0.003 ± 0.0	0

underneath the cages of BF location when viewing the recordings in entirety. The most species abundant family was Sparidae (9 species, 37.5% of overall species richness). Two families, Carrangidae and Serranidae, contributed to species richness with 2 species each, while all other families were represented by a single species (Table 1).

Species richness was the highest near the sea bottom and only bogue, *Boops boops*, and horse mackerel, *Trachurus* sp., were seen at all depths. Downward trend of species richness was noticeable with increasing distance from farm cages, where the largest number of species (22) was recorded at F locations, slightly fewer at MC (16), and yet fewer at C (12) locations. The diversity of fishes was fairly constant between seasons with 20 species observed during the CS and 19 during the WS period. Altogether, 20 species appeared at the BF location and 17 at the UF location.

The recordings were dominated by a relatively small number of species throughout the study. Bogue, *B. boops*, was the most frequently observed fish, with an overall appearance of 11.6%. Only a quarter of fish species had an occurrence frequency $\geq 5\%$, while most species were observed rather scarcely in the recordings. A third of all recorded species showed variability in occurrence frequency with regard to impact level and were from 2 up to 50 times (as in case of gilthead seabream, *Sparus aurata*) more common at farm locations compared to either the MC or C locations. The majority of species (58.3%) were stratified according to depth and occurred more frequently or exclusively at the sea bottom (Table 1). Pickarel, *Spicara sp.*, were the only taxa to display seasonal variation, and were more common during the cold period of the study and relatively rare in the warm period.

3.2. Variations in species richness and abundance

Both the species richness and total fish abundance showed significant differences in regard with Impact and Depth (ImxDe) interaction, pointing towards depth as a driver of differences (Table 2, Fig. 2). The mean species richness was consistently greater (7.1–32.3 times) near the sea bottom than for any other depth strata of the water column with an overall peak at the sea bottom beneath the tuna fish cages (Fig. 2). Significant differences in species richness for depth were found at F and C, with more conspicuous post-hoc results at F ($p < 0.01$) than in C ($p = 0.04$). Pronounced variance of species richness at the sea bottom of the MC impact level diminished the possibility to detect statistical differences with any of the other depth strata. At all water column depths no differences in the number of fish species were recorded between impact levels, while at the sea bottom, there was a significant decrease in species richness between the F and C impact levels ($p < 0.01$), with middle values recorded at MC ($p > 0.05$).

Generally, the total fish abundance patterns reflected those described for species richness (Table 2, Fig. 2). The highest abundance values were recorded at the F impact level and were on average, depending on the considered depth, 4.6 to 44.4 times higher than those at the MC and C impact levels. Abundances at 3 m and 12 m depth and bottom strata showed comparably high values (Fig. 2) but with higher degree of dispersion for the water column than for the bottom layer. Thereby, pair-wise tests only detected significantly higher abundance at the bottom in comparison to the 25 m stratum. Comparatively, due to their pronounced heterogeneity, water column abundances of the F impact level could not be statistically separated from the much lower values at the corresponding depths of the MC and C impact levels. At the bottom, a significant decrease in total fish abundance was present between the end impact levels, F and C, whereas no significant differences were observed between MC and either the F or C impact level (Fig. 2, Table 2). Throughout the study bogue, *B. boops*, was the most abundant fish species regardless of impact level, depth or season of sampling, making up 77.2% of the overall fish abundance observed in the video recordings. Only four other species - two-banded sea bream, *Diplodus vulgaris*, axillary seabream, *Pagellus acarne*, pickarel, *Spicara sp.* and gilthead seabream, *Sparus aurata* - accounted for $\geq 2\%$ each to the overall abundance, while the individual contribution of the majority of recorded fish species was almost negligible.

3.3. Variations in fish assemblage composition and abundance structure

As for univariate analysis, significant ImxDe term for the multivariate data sets of assemblage presence/absence and abundance structure was detected (Table 3). Pairwise comparisons revealed that F and MC bottom assemblages significantly differed from the rest of the water column, while for control (C) locations, no such separation of bottom strata was found (Table 3). Assemblages of any of the water column depths, both within and across impact levels, had comparable composition and structure. Between-impact variability was significant at the bottom. F and MC assemblages showed common patterns of species presence/absence, while the composition recorded at C locations was statistically different from the remaining two impact levels. In terms of abundance assemblages showed a more pronounced structuring with each impact level differing from one another.

The CAP plot of site centroids (Fig. 3) revealed a clear pattern to further corroborate that bottom assemblages from different impact levels were distinguishable from one another, especially those from sites directly beneath the tuna fish farm cages, and notably more so for species abundance structure than for composition. Water column samples, either for composition or abundance, always tended to cluster together.

Vector overlay of the CAP plot (Fig. 3) highlighted the individual fish species characterising the observed differences of the assemblages at the F, MC and C impact levels. Occurrence and abundances of all discriminating species increased closer to tuna fish farms and likewise over the sea bottom. Shift in composition and abundance structure of fish assemblages is strongly associated with the occurrence and abundance dynamics of several gregarious species, primarily bogue, *B. boops*, but also saddled seabream, *Oblada melanura* and pickarel, *Spicara sp.* In addition to these schooling species, the two-banded seabream, *D. vulgaris*, gilthead seabream, *S. aurata*, axillary seabream, *P. acarne*, common pandora, *Pagellus erythrinus* and the mullets, *Mullus sp.* were important in discriminating the assemblages of the different impact levels, as well as the bottom layer from the rest of the water column.

3.4. Variations in proxy measures of fish fitness status

Fish length explained a significant proportion of the variability in fitness status indices of farm associated and wild bogue, *B. boops*, and gilthead seabream, *S. aurata* (Table 4). There was still a significant effect of the Impact and Season interaction term (ImxSe, $p < 0.01$) on the residual variation for any of the analysed indices, excluding just one of the parasite load indices (Table 4, Fig. 4). For Kn seasonal fluctuations were more pronounced (Table 4) and both bogue and seabream were generally better conditioned in the warmer part of the year. Farm associated and wild fish displayed relatively similar patterns of Kn through seasons. However, in summer, bogue and seabream caught in the proximity of tuna fish farms had a significantly higher average Kn (1.06–1.08 times, Fig. 4) than their wild counterparts. This was also true in winter for seabream only (1.14 times greater Kn in farm compared to wild specimens). HI values of wild seabream were seasonally stable throughout the year. From spring to autumn, the HI values of wild seabreams were on average 1.28 to 1.52 times higher and statistically different from values recorded for farm associated specimens, while in winter there was no significant difference. The effect of season from spring to autumn was also observed for bogue, though unlike seabream, farm associated bogue had a 1.23 to 1.48 times higher average HI than wild bogue. GI differed significantly between farm associated and wild specimens of both species during all seasons (Table 4). GI values were consistently > 1.5 times greater in seabream and bogue collected around tuna fish farms compared to wild fish (Fig. 4). The only exception is winter season for bogue when a milder

Table 2

Summaries of PERMANOVAs on total fish species richness (SR) and abundance (SA) testing for the effects of tuna fish farm impact (Im) under Farm (F) vs. Mid-control (MC) vs. Control (C) impact conditions, over spatial scales of Locations (Lo) and Sites (Si), at different depth levels (De, 3 m, 12 m, 25 m and bottom) and over temporal scales of Season (Se) and Time (Ti).

Source of variation	df	SR		SA	
		MS	F	MS	F
Im	2	83.69	10.06 ***	152.41	2.85 *
Lo	1	4.88	1.14	76.20	1.85
Se	1	5.15	0.37	14.15	0.74
De	3	261.81	40.50 ***	66.60	5.38 **
Si(Lo)	2	7.15	1.89	24.25	5.03
Ti(Se)	2	13.36	30.23	42.37	2.43
ImxLo	2	5.49	0.93	77.63	1.34
ImxSe	2	4.06	0.62	1.14	0.15
ImxDe	6	41.58	5.98 ***	15.17	2.25 **
LoxSe	1	1.76	0.34	0.69	0.10
LoxDe	3	2.14	1.24	9.35	1.81
SexDe	3	4.75	0.62	11.83	3.89 *
ImxSi(Lo)	4	8.12	2.82	33.25	9.63 **
ImxTi(Se)	4	2.91	3.48	21.44	0.78
LoxTi(Se)	2	0.44	0.12	17.44	3.61
Si(Lo)xSe	2	15.97	4.23	35.39	7.34
Si(Lo)xDe	6	4.42	0.97	2.60	0.68
Ti(Se)xDe	6	4.35	4.57	3.89	0.84
ImxLoxSe	2	4.98	3.46	3.13	0.17
ImxLoxDe	6	1.75	0.37	12.45	1.50
ImxSexDe	6	1.91	0.49	6.16	1.24
LoxSexDe	3	4.85	1.12	0.34	0.30
Si(Lo)xTi(Se)	4	3.78	14.46 ***	4.82	10.37 ***
ImxLoxTi(Se)	4	0.84	0.29	27.18	7.87 **
ImxSi(Lo)xSe	4	1.43	0.50	13.39	3.88
ImxSi(Lo)xDe	12	6.46	4.05 ***	3.97	1.01
ImxTi(Se)xDe	12	5.64	2.15	4.52	0.65
LoxTi(Se)xDe	6	0.95	0.21	4.63	1.21
Si(Lo)xSexDe	6	7.54	1.66	9.09	2.39
ImxLoxSexDe	6	3.70	1.14	5.97	0.50
ImxSi(Lo)xTi(Se)	8	2.88	11.02 ***	3.45	7.42 ***
Si(Lo)xTi(Se)xDe	12	4.54	17.36 ***	3.80	8.17 ***
ImxLoxTi(Se)xDe	12	2.63	1.65	6.87	1.75
ImxSi(Lo)xSexDe	12	2.01	1.26	12.88	3.28 **
ImxSi(Lo)xTi(Se)xDe	24	1.59	6.10 ***	3.92	8.43 ***
Res	960	0.26		0.46	

Pair-wise test for ImxDe interaction

SR	Impact		Depth	SA	Impact		Depth
3 m	F = MC = C	F	bottom > 25 m = 12 m = 3 m	3 m	F = MC = C	F	bottom > 25 m; 25 m = 12 m = 3 m; bottom = 12 m = 3 m
12 m	F = MC = C	MC	bottom = 25 m = 12 m = 3 m	12 m	F = MC = C	MC	bottom = 25 m = 12 m = 3 m
25 m	F = MC = C	C	bottom > 25 m; 25 m = 12 m = 3 m; bottom = 12 m = 3 m	25 m	F = MC = C	C	bottom = 25 m = 12 m = 3 m
bottom	F = MC; F > C; MC = C			bottom	F = MC; F > C; MC = C		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

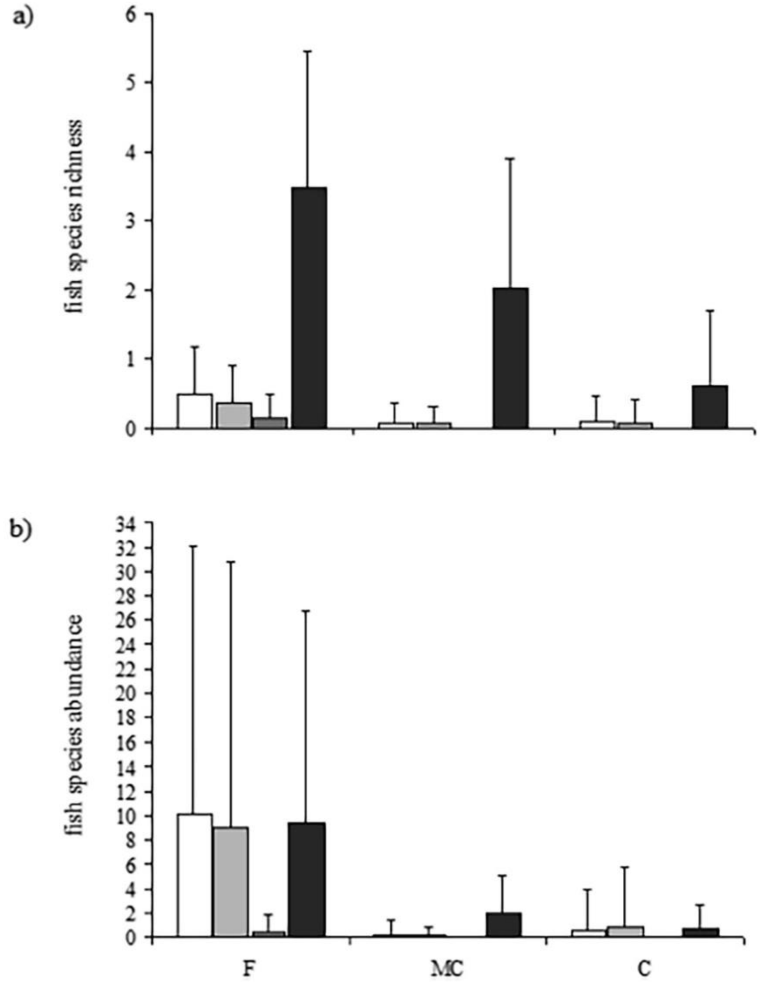

Fig. 2. Mean (+SD) fish species richness (a) and total fish abundance (b) per 1090 m^3 at different depth strata (3 m; 12 m; 25 m; bottom) of tuna fish farm (F), mid-control (MC) and control (C) locations.

inverse difference was found – 1.3 higher GI values for wild specimens in comparison to the ones associated with farms. The GI of both analysed fish species was highest in seasons corresponding to their spawning period, i.e. autumn for seabream and spring for bogue.

Upon macroscopic inspection for the presence of parasites, none of the seabream specimens, either farm-associated or wild, were found to be infected with any parasite taxa. The parasitic assemblage of farm associated bogue and their non-associated wild counterparts consisted of three taxa – two external parasites: cymothoid isopods of the genus *Ceratothoa* and the copepod species *Lernaeolophus sultanus*, both of which were found attached exclusively to the bucal cavity of the fish host, and one internal parasite: the nematode *Anisakis simplex*.

Of the three taxa found parasiting bogue, anisakis had the highest overall prevalence (42.6%) and fish caught in the vicinity of tuna fish farms (69.0%) had a much higher infection rate than the wild counterparts (3.2%). Statistically significant differences were also detected between farm associated and wild bogue for the mean abundance of anisakis individuals per fish host (Table 4, Fig. 4). Abundances of anisakis in farm associated bogue were particularly prominent in the colder part of the year (winter and spring, Table 4). Summer was the only season when a low abundance of 0.4 ± 1.3 anisakis individuals was recorded in wild bogue specimens, while no anisakis were observed in bogue caught far off tuna fish farms in any of the other seasons.

The overall prevalence of parasitic isopods was 8.8%, and farm associated bogue were more infected (13.5%) than their wild counterparts (1.8%). In general terms, the mean abundances of parasitic isopods per fish host exhibited a similar pattern of seasonal variation as already described for anisakis (Fig. 4). Infection with parasitic copepods was generally very low with infection prevalence of only 0.9%. Only six individuals of parasitic copepod were recorded on five farm-associated and one wild bogue out the total 704 inspected. Variations found for the mean number of copepod individuals per fish host were mostly accounted for by fish host size, while impact or season associated differences were negligible (Table 4). All parasitic copepods were observed in summer or autumn with similar abundances, while no bogue specimens, farm or wild, harboured this parasite in the colder seasons (winter and spring; Fig. 4).

4. Discussion

4.1. Semi-offshore tuna farms and spatial distribution of wild fish

The suite of potential ecological effects tuna fish farms can have on wild fish residing in their vicinity is still largely undocumented. This study has provided evidence of the substantial capacity of tuna fish farms to aggregate the surrounding wild fish assemblages. Significantly higher species diversity and abundance of fish at both studied farms compared to any of the control locations clearly indicate that tuna fish

Table 3

Summaries of PERMANOVAs on fish assemblage composition (FAC) and abundance structure (FAS) testing for the effects of tuna fish farm impact (Im) under Farm (F) vs. Mid-control (MC) vs. Control (C) impact conditions, over spatial scales of Locations (Lo) and Sites (Si), at different depth levels (De, 3 m, 12 m, 25 m and bottom) and over temporal scales of Season (Se) and Time (Ti).

Source of variation	df	FAC		FAS	
		MS	F	MS	F
Im	2	30534.00	3.79 *	13956.00	3.02 *
Lo	1	9732.50	2.32	8634.60	2.62
Se	1	10752.00	1.09	5867.30	0.96
De	3	73517.00	8.23 **	26738.00	4.46 **
Si(Lo)	2	2930.10	0.99	2430.70	1.42
Ti(Se)	2	7482.60	2.95	5638.30	3.70
ImxLo	2	8641.00	1.17	8268.20	1.72
ImxSe	2	3803.30	0.94	1152.60	0.89
ImxDe	6	13308.00	2.52 **	6897.10	2.66 ***
LoxSe	1	4664.10	0.85	2087.60	0.76
LoxDe	3	5547.10	1.78 *	3482.10	2.15 **
SexDe	3	7261.10	1.21	3911.80	1.17
ImxSi(Lo)	4	5943.00	2.87 *	3202.30	3.32 **
ImxTi(Se)	4	2658.00	0.84	1737.60	0.79
LoxTi(Se)	2	2532.50	0.86	1523.90	0.89
Si(Lo)xSe	2	6429.70	2.17	3457.30	2.02
Si(Lo)xDe	6	1868.00	0.88	1146.60	0.96
Ti(Se)xDe	6	3679.60	1.51	2736.50	2.67 *
ImxLoxSe	2	4758.10	1.24	2020.30	0.85
ImxLoxDe	6	3116.70	0.79	2365.90	1.21
ImxSexDe	6	3346.40	1.01	1673.30	0.85
LoxSexDe	3	4319.70	1.06	1479.40	0.77
Si(Lo)xTi(Se)	4	2957.30	14.24 **	1710.10	13.14 ***
ImxLoxTi(Se)	4	3181.60	1.53	2175.20	2.25
ImxSi(Lo)xSe	4	2344.50	1.13	1303.80	1.35
ImxSi(Lo)xDe	12	3163.20	2.19 **	1391.60	1.62 *
ImxTi(Se)xDe	12	3194.90	1.24	1515.20	1.19
LoxTi(Se)xDe	6	2432.90	1.15	1024.20	0.86
Si(Lo)xSexDe	6	3658.40	1.73	2407.40	2.02 *
ImxLoxSexDe	6	2684.00	0.95	1947.60	1.01
ImxSi(Lo)xTi(Se)	8	2073.00	9.98 ***	962.48	7.40 **
Si(Lo)xTi(Se)xDe	12	2111.80	10.17 ***	1190.20	9.14 ***
ImxLoxTi(Se)xDe	12	2581.70	1.78 **	1271.30	1.48
ImxSi(Lo)xSexDe	12	1777.90	1.23	1498.90	1.74 **
ImxSi(Lo)xTi(Se)xDe	24	1446.30	6.97 ***	858.75	6.60 ***
Res	960	207.63		130.10	

Pair-wise test for ImxDe interaction

FAC	Impact		Depth	FAS	Impact		Depth
3 m	F = MC = C	F	bottom ≠ 25 m = 12 m = 3 m	3 m	F = MC = C	F	bottom ≠ 25 m = 12 m = 3 m
12 m	F = MC = C	MC	bottom ≠ 25 m = 12 m = 3 m	12 m	F = MC = C	MC	bottom ≠ 25 m = 12 m = 3 m
25 m	F = MC = C	C	bottom = 25 m = 12 m = 3 m	25 m	F = MC = C	C	bottom = 25 m = 12 m = 3 m
bottom	F = MC ≠ C			bottom	F ≠ MC ≠ C		

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

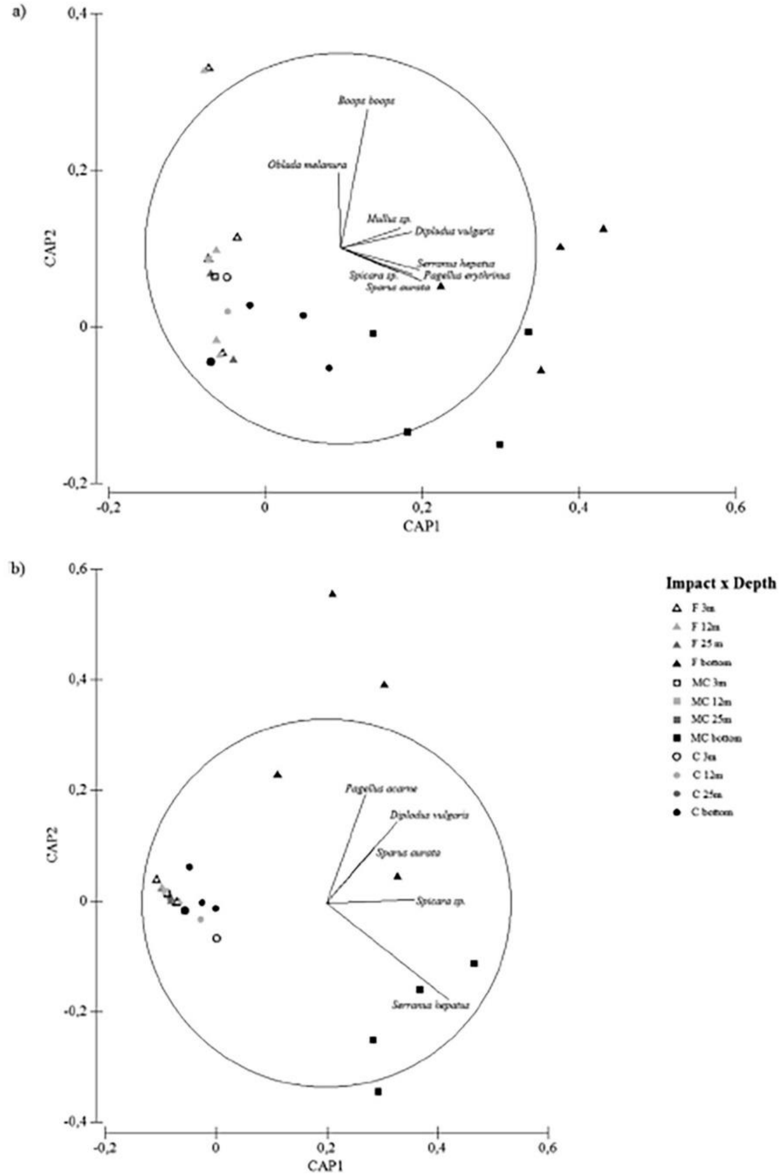

Fig. 3. Canonical Analysis of Principal Coordinates (CAP) ordination of fish assemblage: a) composition and b) abundance structure showing the interaction between impact level (tuna fish farm (F); mid-control (MC) and control (C)) and depth. Vector overlays of strongly correlated fish species ($r > 0.30$) are presented. Symbols in the ordination represent group centroids for each sampling site during both warm and cold season of sampling.

farms provide an attractive habitat for associated wild fish populations.

Aggregations of farm associated wild fish seem to be strongly dominated by a relatively small number of species (Bacher et al., 2012; Ballester-Moltó et al., 2015; Boyra et al., 2004; Dempster et al., 2005, 2009, 2010; Fernandez-Jover et al., 2008; Valle et al., 2007). In the Mediterranean particularly, bogue has been identified as a significant and abundant component of aggregated assemblages around fish farms (Arechavala-Lopez et al., 2011; Bacher et al., 2012; Ballester-Moltó et al., 2015; Dempster et al., 2002, 2005; Fernandez-Jover et al., 2008; Šegvić Bubić et al., 2011; Valle et al., 2007). At the studied tuna fish farms, bogue can be considered a resident species due to the year round presence in high abundances (representing on average 77% of overall number) without displaying detectable seasonal shifts. A high degree of residency was already noted at these farms in a previous research, although Šegvić Bubić et al. (2011), focusing exclusively on water column near the surface, observed shoal dominance during the warmer part of the year. Curiously, also opposite seasonal aggregation patterns with higher abundances in the cold part of the year have been recorded for bogue in other Mediterranean studies (Bacher et al., 2012; Fernandez-Jover et al., 2008; Valle et al., 2007). Besides displaying large, highly abundant and seasonally stable aggregations during the year of this study, another specificity of the observations was that bogue, habitually a pelagic planktivorous species (Jardas, 1996), was observed 1.5-times more frequently over the bottom than at the surface, and a considerable proportion of specimens were invariably keeping close to the bottom. The peculiar depth stratification of bogue at tuna fish farms likely reflects the vertical differences in food availability, as discussed further in the paragraph below. A further common feature emerging from this and other Mediterranean studies (Arechavala-Lopez et al., 2011; Bacher et al., 2012; Ballester-Moltó et al., 2015; Dempster et al., 2002, 2004, 2005; Fernandez-Jover et al., 2008; Šegvić Bubić et al., 2011; Valle et al., 2007) is that Sparidae are consistently observed as one the most species rich families around both types of fish farms. The attraction effect is also usually manifested through an overall more diverse fish assemblage at farms compared to control locations (Bacher et al., 2012; Boyra et al., 2004; Dempster et al., 2002; Ozgul and Angel, 2013; Šegvić Bubić et al., 2011; Valle et al., 2007), which was also the pattern detected in this study. A third of all the recorded fish species were significantly more common in close proximity to the farms, e.g. seabream, *S. aurata*, occurred as much as 50 times more frequently in the recordings at farms than at controls. Furthermore, a much more species rich assemblage resided near the bottom than in any of the analysed water column depths, which is consistent with few other studies (Bacher et al., 2012; Dempster et al., 2005, 2009). Higher fish diversity over the bottom than in the water column is a general feature of littoral fish assemblages, reflecting the differences in habitat heterogeneity. Structurally more complex bottom habitats are able to sustain more diverse and numerous fish communities than the homogeneous pelagic

Table 4
PERMANOVA table of fitness status indices for a) *Sparus aurata* and b) *Boops boops* testing for differences between impact levels (Im; farm (F) vs. wild (W)) and seasons (Se; Spring (Spr), Summer (Sum), Autumn (Aut), Winter (Win)) of sampling while taking into account fish size (TL, total length) as a covariate.

a) *Sparus aurata*

Source of variation	df	Kn		HI		GI	
		MS	F	MS	F	MS	F
TL	1	0.05	9.07 **	1.81	6.80 **	104.99	62.09 ***
Im	1	0.01	3.09	8.35	31.41 ***	14.55	8.61 **
Se	3	0.27	43.72 ***	1.26	4.75 **	132.41	78.31 ***
ImxSe	3	0.15	23.10 ***	3.34	12.55 ***	30.41	17.98 ***
Res	611	0.01		0.27		1.69	

Pair-wise test for ImxSe interaction

	Kn	**HI**	**GI**
Impact			
Spring	F = W	F < W	F > W
Summer	F > W	F < W	F > W
Autumn	F = W	F < W	F > W
Winter	F > W	F = W	F > W
Season			
Farm	Spr < Sum = Aut = Win	Spr = Sum = Aut < Win	Spr = Sum < Win < Aut
Wild	Spr = Sum = Win < Aut	Spr = Sum = Aut = Win	Spr = Sum < Aut = Win

b) ***Boops boops***

Source of variation	**df**	**Kn**		**HI**		**GI**	
		MS	**F**	**MS**	**F**	**MS**	**F**
TL	1	0.01	0.48	12.46	95.35 ***	20.87	100.7 ***
Im	1	0.00	0.001	0.40	3.08	5.41	26.12 ***
Se	3	0.46	35.91 **	1.96	15.01 ***	49.06	236.8 ***
ImxSe	3	0.08	6.54 ***	1.06	8.11 ***	11.24	54.24 ***
Res	695	0.01		0.13		0.21	

Pair-wise test for ImxSe interaction

	Kn	**HI**	**GI**
Impact			
Spring	F = W	F = W	F > W
Summer	F > W	F = W	F > W
Autumn	F = W	F = W	F > W
Winter	F = W	F = W	F < W
Season			
Farm	Spr < Sum = Win < Aut	Sum > from all other; Spr = Aut; Spr = Win; Aut > Win	Spr > Sum > Win > Aut
Wild	Spr = Sum < Aut = Win	Spr < Sum = Aut = Win	Spr = Win > Sum = Aut

Source of variation	**df**	**anisakis infestation**		**isopod infestation**		**copepod infestation**	
		MS	**F**	**MS**	**F**	**MS**	**F**
TL	1	9728.8	129.9 ***	0.06	0.36	0.05	5.52 **
Im	1	2312.3	30.89 ***	11.86	65.9 ***	0.00	0.11
Se	3	850.38	11.36 ***	2.08	11.57 ***	0.01	1.46
ImxSe	3	439.68	5.87 ***	2.55	14.18 ***	0.01	0.87
Res	695	74.863		0.18		0.01	

(continued on next page)

Table 4 (*continued*)

a) *Sparus aurata*							
Source of variation	df	Kn		HI		GI	
		MS	F	MS	F	MS	F
Pair-wise test for ImxSe interaction							
		anisakis infestation		**isopod infestation**			
Impact							
Spring		F > W		F > W			
Summer		F > W		F = W			
Autumn		F > W		F > W			
Winter		F > W		F > W			
Season							
Farm		Spr = Win > Aut; Spr > Sum; Win = Sum; Sum = Aut		Spr = Win > Sum = Aut			
Wild		Sum ≥ Aut = Win = Spr		Sum ≥ Aut = Win = Spr			

*p < 0.05; **p < 0.01; ***p < 0.001.

environment (Anderson and Millar, 2004; Garcia-Charton and Perez-Ruzafa, 1998; Garcia-Charton et al., 2004; Guidetti, 2000; Kaiser et al., 1999). Sea cages at fish farms add structure to the water column, analogous to the large fish attraction devices (Dempster et al., 2002; Dempster and Taquet, 2004), further enhancing the attraction of fish owing to the large amounts of waste feed available at the farms (Ballester-Moltó et al., 2017; Tuya et al., 2005, 2006).

The observed aggregation effect persisted throughout the year of the research. This stability implies that tuna fish farms represent perseveringly important habitat for wild fish, as has already been put

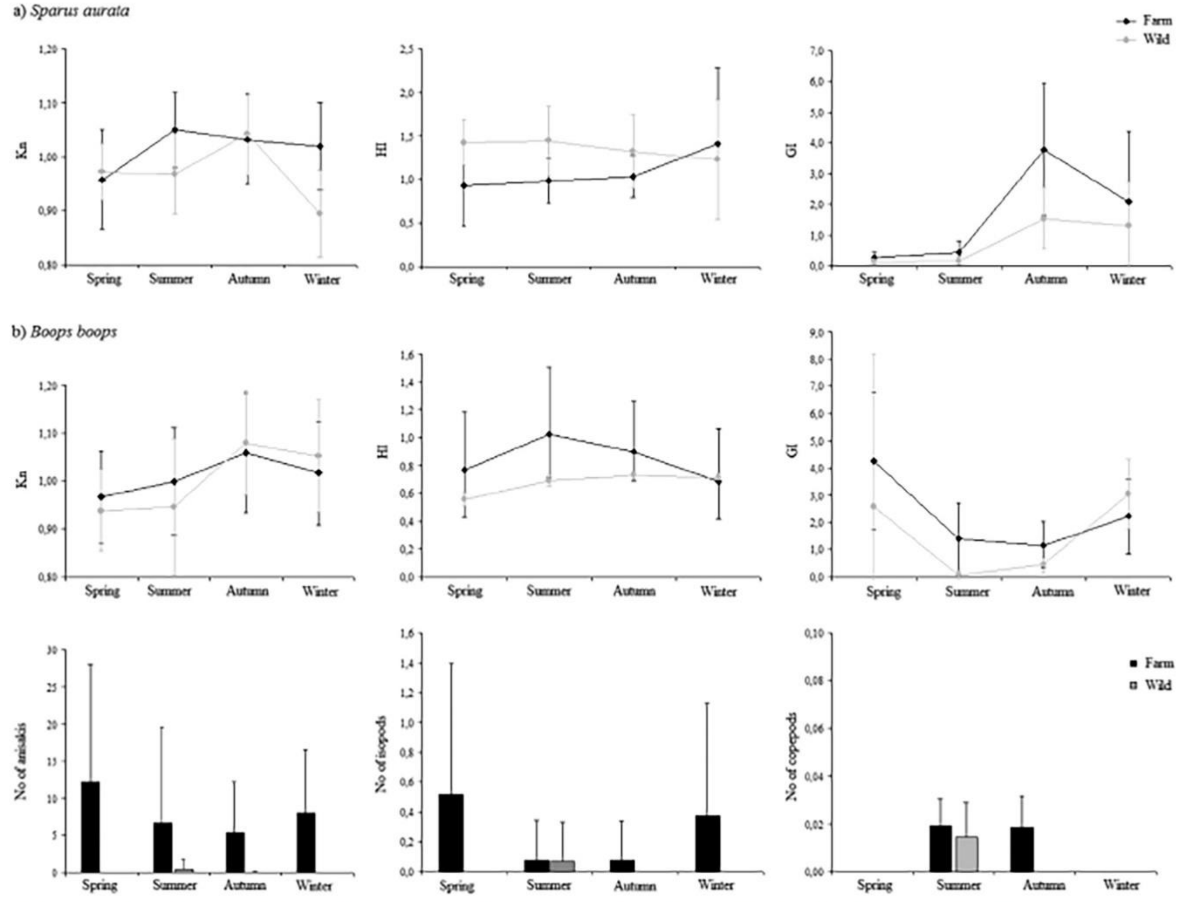

Fig. 4. Fitness status indices of farm-associated and wild a) *Sparus aurata* b) *Boops boops*. Kn = relative body condition index; HI = hepatosomatic index; GI = gonadosomatic index and parasite loads shown as mean abundance (No + SD) of parasite specimens per fish.

forward by Dempster et al. (2002, 2004) for seabream/seabass coastal farms. However, pronounced univariate and multivariate heterogeneity was recorded on a small temporal and spatial scale, among random sampling times within season and among sampling sites. The assemblages at farm locations were characterized by a high and more varying number of species and abundances of fish, as well as by greater variation in composition and structure, while mid and control locations had consistently lower numbers of species and individuals present. Pronounced small-scale temporal and spatial variabilities are in fact a typical and naturally inherent feature of littoral fish assemblages reflecting the patchiness of fish distribution, particularly of gregarious species (Guidetti, 2000). Contrary to small-scale heterogeneity, there were no detectable seasonal variations. Seasonally stable aggregations associated with fish farms were also found in the sub-tropical region of the Canary Islands (Boyra et al., 2004), while in temperate Mediterranean regions temporal stability of aggregations, both in relative size and species composition, was observed only over periods of several weeks to months (Dempster et al., 2002, 2004). At a seasonal scale, temporal patterns typically exhibit significant fluctuations in temperate regions and most studies detect seasonality, in addition to increased abundance and altered fish assemblage composition as a result of aquaculture (Arechavala-Lopez et al., 2015a; Bacher et al., 2012; Fernandez-Jover et al., 2008; Valle et al., 2007). Focusing on pelagic assemblages in a year round study at the same tuna fish farms as assessed here, Šegvić Bubić et al. (2011) observed significant fluctuations in abundance and composition according to seasons. In this study, when wild fish aggregations were examined throughout the water column, depth appeared to be a stronger determinant factor, exceeding the influence of season.

Information on spatial variability with depth of fish residing in the vicinity of farm cages is relatively scarce and not so straightforward to interpret. It is clear that depth is a strong factor influencing the abundance and structure of wild fish aggregations, but where the aggregated fish mainly distribute in the water column varies. Dempster et al. (2005) after the study of five fish farms at four different depths, concluded that there is not a common inter-farm vertical distribution pattern of fish aggregated assemblages. Our results, according to all the analysed parameters – species richness, abundance of fish, assemblage composition and structure, invariably show that the most pronounced impact of semi off-shore tuna farming is seen at the bottom layer. At all the water column depths, univariate and multivariate patterns of fish assemblages were comparable between impact levels. The increased fish aggregations found in the bottom strata of semi-offshore cages as opposed to considerable pelagic aggregations at coastal cages (Arechavala-Lopez et al., 2015a; Bacher et al., 2012; Boyra et al., 2004; Dempster et al., 2002, 2009; Goodbrand et al., 2013; Özgül and Angel, 2013; Šegvić Bubić et al., 2011) might be due to differences either in practices, setting or environmental characteristics of the farming sites (Vita et al., 2004). The potential drivers of differences in fish assemblages between farms can be reflected in: (i) substrate type and complexity; (ii) distance from the coast, again related to the proximity to more complex habitats of seagrass meadows and rocky reefs; (iii) depth; (iv) currents; (v) wave exposure; (vi) composition of fish communities in surrounding natural habitats; (vii) proximity to important fish migration routes, and (viii) fishing pressure (Arechavala-Lopez et al., 2011; Bacher et al., 2012, 2015; Boyra et al., 2004; Dempster et al., 2002, 2005, 2009; Fernandez-Jover et al., 2008; Tuya et al., 2005). Thus, knowing the size and nature of aggregations of a single farm cannot straightforwardly be used to predict how will wild fish associate with other farms (Dempster et al., 2005; Fernandez-Jover et al., 2008).

The distinction between the two farming models is, however, primarily based on the type of feed used, i.e. defrosted baitfish vs. pellets. Various baitfish used to feed reared tuna differ considerably from pellets fed to finfish at coastal farms in their nutrient content and leachability, food size and settling velocities (Aguado-Gimenez and Garcia-Garcia, 2005; Fernandes et al., 2007; Vassallo et al., 2006). Whether associated wild fish will be predominantly pelagic or demersal is largely determined by where in the water column the feed lost from the cages is primarily available (Bacher et al., 2012; Ballester-Moltó et al., 2017; Dempster et al., 2005). Vita et al. (2004) showed that wild fish are able to withdraw up to 80% of the particulate organic matter leaving the cages, while Ballester-Moltó et al. (2017) put an estimate at only 17.75% pointing that solid waste removal by wild fish was higher close to the bottom of the cages. The consumption of waste feed by wild fish in the water column also occurs at tuna fish farms where, bogue, *B. boops*, and, garfish, *B. belone*, were seen foraging extensively on pieces of baitfish around the cages (Šegvić Bubić et al., 2011). The proportion of baitfish lost through the tuna rearing cages is generally unknown. A rough estimate of 4–23% loss exists for the southern bluefin tuna, *Thunnus maccoyii* (Bruce, 1997 cited in Fernandes et al. (2007)), while the amount of uneaten baitfish that actually reaches the bottom beneath the cages is still completely unexplored. Presumably, much of the baitfish falling through the cages settles on the seafloor, and hence a stronger attraction effect is seen.

Regarding the spatial extent of farming impact in the horizontal direction, this was found largely to be constrained to the close proximity of sea cages. Even at bottom where the farm impact is conspicuous, most of the attraction effect diminishes by the first mid-control locations, 500 m away. Dempster et al. (2010) observed the majority of fish concentrated as close as within the first 25 m of coastal salmon cages. The nearest control sites used by Bacher et al. (2012) at Mediterranean tuna fish farms were situated only 100 m away at the edge of the cages, and the results also support the conclusion that aggregation effect does not follow a distance gradient, but rather shows an abrupt decline. The short horizontal extension of the fish farming attraction effect is similar to that found for the spillover effect or export of adult fish from MPAs (Harmelin-Vivien et al., 2008).

4.2. *Semi-offshore tuna farms and fish condition*

The aggregative behaviour of wild fish around fish farms has been primarily attributed to permanent and increased availability of food (Tuya et al., 2006). Dietary shifts away from natural foods towards this easily accessible, high energy trophic resource can lead to an overall higher fitness status of fish residing in the vicinity of aquaculture installations (Fernandez-Jover et al., 2007, 2008). In present study, higher indices of condition in two focal fish species, seabream and bogue, were detected near the farms, confirming that farm associated specimens tend to be better nourished than their wild counterparts caught distant from farms. Variations in Kn body condition showed a more pronounced seasonal pattern compared to farming impact related differences. Nevertheless, in the warmer part of the year, bogue and seabream keeping close to farms displayed significantly higher levels of body condition than wild specimens unaffected by farm vicinity. Seasonal variation observed in the energy status of seabream and bogue is easily related to reproductive cycle and seawater temperature related feeding intensity (Lambert and Dutil, 1997). Lower HI observed in farm associated seabreams for most of the year, may indicate that seabream under abundant and lipid enriched food conditions adopt a maximization strategy of reproductive growth over energy storage maximization (Fernández-Palacios et al., 2011). The allocation of energy reserves among different organs is species-specific (Shulman and Love, 1999) and crucial for the distribution of energy to various physiological processes in fish are the availability and quality of food (Adams et al., 1982). Fernandez-Jover et al. (2007) found that liver condition of Mediterranean horse mackerel, *Trachurus mediterraneus* aggregated at coastal finfish farms was higher in control, natural populations. These findings were attributed to the ingestion of hormonally active compounds in waste feed, considered capable of modifying liver weight due to the activation of metabolising enzymes. Contrary to seabream, the hepatic condition of bogue was elevated in specimens aggregating around farms compared to those caught further away. However, HI values of farm associated bogue were also characterized by a high degree of variability which could be due to differing residence time of individuals at farms prior to capture (Fernandez-Jover et al., 2007). Further studies on the physiological effects of fish farming on associated wild fish species are needed, in particular on how the surplus energy is distributed into growth, reproduction or storage and how these processes vary according to the quantity and quality of available food. Increased energy from the farm trophic subsidy seems to be invested most in gonadal development and maturation, as indicated by the highly elevated GI values throughout the year in both investigated fish species. Increased condition of gonads typically corresponds to enhanced reproductive output (Izquierdo et al., 2001), though for fish relying on the trophic resources provided by fish farms, the quality of feed relative to natural diet may also play a role in whether the higher GI ultimately translates to higher spawning success. Different authors (Arechavala-Lopez et al., 2011; Fernandez-Jover et al., 2007, 2011; Izquierdo-Gómez et al., 2014; Uglem et al., 2014) found that the composition of stored lipids in fish aggregating around coastal farms reflected the lipid composition of pellets. Pelleted fish feeds contain high proportions of terrestrial derived fatty acids, and low proportions of highly unsaturated omega-3 fatty acids, which are believed to be crucial for optimal development of fish egg and larvae (Lanes et al., 2012; Laurel et al., 2010; Salze et al., 2005). It is still unclear whether this altered fatty acid composition of farm associated fish has negative side effects for the success of spawning. As tunas are fed exclusively with baitfish composed of a variety of small pelagic species rich in omega-3 and 6 fatty acids (Katavić et al., 2003; Ottolenghi, 2008), reliance on this trophic subsidy might actually have a positive influence on reproductive physiology of wild fish residing in the vicinity of semi-offshore tuna fish farms.

4.3. *Semi-offshore tuna farms and parasite loads*

Parasitic infestation, as a reflection of fish health and fitness, was documented for farm associated and wild bogue, while all analysed seabream were parasite free. Parasitic assemblage of bogue consisted of three taxa – two ectoparasites (*Ceratothoa sp.* cymothoid isopod and *Lernaeolophus sultanus* copepod) and one endoparasite (nematode *Anisakis simplex*). A substantially high proportion of parasitized bogue in comparison to none of the seabreams infected might be related to different modes of feeding and ensuing anatomical differences of the oral cavity, teeth and digestive tract. Both analysed fish species have been characterized as opportunistic feeders capable of strongly adapting their feeding behaviour to the food items available in the habitat. Although bogue is known to have a preference for plant material and is commonly a microphagic carnivore (Bell and Harmelin-Vivien, 1983; Jardas, 1996) around tuna fish farms, during previous (Šegvić Bubić et al., 2011) and this research, bogue were frequently observed actively feeding on pieces of baitfish. Seabream, on the other hand, is assumed to rely more on the shellfish growing on mooring structures and ropes, which corresponds to their usual prey in natural habitat surroundings (Jardas, 1996; Pita et al., 2002). Although stomach content analyses are needed to assert these assumptions, if shown true it would render the seabream less susceptible to infestation by anisakids, as small pelagics used as baitfish have all been confirmed to be moderately to highly infected paratenic hosts (Mladineo and Poljak, 2014). Furthermore, as a carnivore, seabream has a strong, muscular stomach and relatively short intestines, which reduces the chances of anisakis larval penetration through the walls of the alimentary tract to the viscera where they commonly remain encysted in the host (Mladineo and Poljak, 2014). In support of these claims are extremely rare reports of anisakid infections in wild seabream (Abollo et al., 2001; Kalay et al., 2009). Anisakis was highly prevalent in bogue, and significantly more so in the farm associated than in the wild specimens. The observed farm effect is likely related to differences in diet, with more heavily infected farm associated bogue relying on baitfish as an important trophic resource. Furthermore, anisakid infestation in bogue showed seasonal variation, with opposite patterns in wild and farm associated specimens. Summer was the only season in which a low abundance of anisakis individuals was recorded in wild bogue, corresponding to the temporal oscillations in the intensity of anisakid infections described for other regions and fish hosts (Chou et al., 2011; Mladineo and Poljak, 2014; Strømnes and Andersen, 2000). Such anisakid dynamics, with peak values in warmer seasons, has been associated with seasonal fluctuations of abiotic and biotic environmental conditions, particularly with the bloom of zooplankton as the first link in the complex anisakis life cycle (Strømnes and Andersen, 2000). Atypical higher levels of infection during colder seasons were observed in bogue aggregating around tuna fish farms. A possible explanation is that when other natural food items are at their lowest, baitfish likely comprises the greatest proportion of the diet and, hence, the intensity of anisakis infection increases. As bogue is a commercial species possible implications of elevated anisakis infestation for human health should be considered. The research linking consumption of infected fish and human health consequences mostly relates to small pelagics, i.e. anchovies and sardines, for which in the Mediterranean there is a tradition of eating marinated or salted. However, if fish is adequatelly thermally processed prior to consumption the health risk of anisakiasis is supposed to be negligible (Mladineo and Poljak, 2014).

Trophic resources in the vicinity of farms are also increased due to the abundance of fouling organisms on cage nets and mooring ropes. Bogue have previously been observed to actively exploit these aquaculture resources at numerous Mediterranean coastal fish farms (Arechavala-Lopez et al., 2011). Grazing of fouling organisms could be the likely pathway of the heightened infestation of farm associated bogue with cymothoid isopods. Cerathothoa isopods are ubiquitous fish parasites, which have been most frequently isolated from bogue (Charfi-Cheikhrouha et al., 2000; Mladineo et al., 2009). Nevertheless, the exact transmission of these ectoparasites in aquaculture surroundings still needs to be investigated. The detected farm effect with bogue caught around tuna cages showing increased isopod prevalence and abundance compared to their wild counterparts, together with a similar pattern of seasonal variation as for anisakis, are strongly indicative of a more specialist feeding behaviour of bogue during colder months when natural pelagic trophic resources are less abundant, while farm related resources are ever-present. The prevalence and abundance of parasitic copepods was much lower than the other two parasites recorded for bogue. Parasitic impact of the observed copepod is largely undocumented, and although it appears from our data that infection levels could be naturally low, research on its life cycle is needed to elucidate the patterns and consequences of infection for fish hosts. Overall, it seems as though parasites have not exerted detrimental effects on bogue general health and fitness status, as farm associated fish with greater parasite loads were also found to have higher body condition and reproductive indices. Still, it should be considered to inspect wild fish at a seasonal basis in order to detect potential disease outbreaks transferred from or towards cultured fish. Congruent with Dempster et al. (2011), we conclude that the strong effect of the trophic subsidy farms provide appears to override any adverse effects of altered parasite loads upon welfare of aggregated wild fish.

5. Conclusions

Taken together our results on the attraction and fitness status of wild fish in the vicinity of semi-offshore tuna fish farms support the view of aquaculture installations as population sources, or high quality habitats enhancing the productivity of coastal fish assemblages. A certain contribution to such positive outcomes is that strict fishing restrictions are in effect within farm leasehold areas in Croatia. Spatial protection from fishing at fish farms has been enacted through the legislation of some countries, e.g. Spain and Norway, but not all, as for example is the case in Australia (Dempster et al., 2004, 2011).

Exclusion of all forms of fishing should be promoted as a standard within leasehold areas, as under no fishing conditions, leashehold areas can act as MPAs (Dempster et al., 2002, 2006). Exclusion from fishing enables farms to fulfil dual objectives of protecting the fish, and enhancing local fisheries through adult biomass export and increased reproductive output (Dempster et al., 2005; Fernandez-Jover et al., 2007). On the other hand, allowing fishing could lead to local overexploitation, with leasehold areas functioning as ecological traps (Dempster et al., 2011). When aggregated around fish farms, fish are particularly vulnerable to capture. Moreover, as farms are highly attractive to wild fish, they would continuously draw fish from surrounding waters into their vicinity, where the increased fishing pressure would drive populations even further down (Arechavala-Lopez et al., 2011; Dempster et al., 2011). Another advantage of having undisturbed attraction of wild fish to sea cage farms is the reduced environmental footprint, primarily through the removal of waste feed i.e. biofilter role (Vita et al., 2004; Uglem et al., 2014). With this research, we have added to the growing body of evidence that, if planned properly, aquaculture and conservation can work in support of each other.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.10.015.

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Morphological and ecophysiological adaptations of wild gilthead seabream *Sparus aurata* associated with tuna farms

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ABSTRACT: Morphological and ecophysiological traits of wild, farmed and wild farm-associated gilthead seabream *Sparus aurata* were used to assess the degree of phenotypic adaptation of the species to their respective environments. Geometric morphometrics revealed clear body shape differentiation amongst the 3 types of populations, whereby significant differences were noted in head profile and the anterior-body region of the fish. Morphological resemblance was recorded among 2 gilthead seabream populations associated with tuna farms, indicating that they share the same phenotypic responsiveness pattern. Adaptation in reproductive investment strategy was observed in individuals from farm-associated origin, displaying the highest gonadosomatic index but lowest condition in comparison to their farmed and wild counterparts. Multinomial logistic regression revealed that sex change from male to female with increasing length was more pronounced in farm-associated fish than in farmed and and wild fish. These findings demonstrate a significant influence of the environmental factors inherent to tuna farms on the phenotypic characteristics of gilthead seabream, providing a basis for further research on the ecological effects of tuna farms on local marine populations.

KEY WORDS: Gilthead seabream · Aquaculture impact · Farm-associated fish · Phenotypic plasticity · Morphology · Fitness

1. INTRODUCTION

The deterministic role of the environment plays a crucial part in the evolution of phenotypes, especially when considering that many individual characteristics are phenotypically plastic due to the ability of a single genotype to produce multiple phenotypes as a function of environmental conditions (Swain & Foote 1999, Sultan & Stearns 2005). Fish body shape is the product of genetic makeup and ontogenetic allometry, where differential phenotypic characteristics may be attributed to environmental influences rather than to genetic differentiation (Swain & Foote 1999, Cadrin 2005). Morphological and physiological traits are often assumed to reflect adaptation to environmental factors such as temperature (Koumoundouros et al. 2009, Loizides et al. 2014, Nikolioudakis et al. 2014), type of food (Dempster et al. 2011, Abaad et al. 2016) and time the organisms have spent in the given environment (Fleming et al. 1994, Rogdakis et al. 2011, Lorenzen et al. 2012, Wringe et al. 2015). The existence of a relationship between phenotypic variation among fish populations and their functional ecology allows researchers to assess the degree to which a population can modify its fitness in relation to its feeding behaviour and habitat use (Leisler & Winkler 1985, West-Eberhard 1989, Costa & Cataudella 2007).

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The attraction effect of fish farms on wild fish populations (Dempster et al. 2005, Sanchez-Jerez et al. 2011, Šegvić-Bubić et al. 2011c, Stagličić et al. 2017) and the ecological and genetic impacts of escapees on wild counterparts (Rogdakis et al. 2011, Šegvić-Bubić et al. 2011a, 2014, 2017, Arechavala-Lopez et al. 2013, Toledo-Guedes et al. 2014a,b) have been widely studied and provide the basis for recommendations to manage escape scenarios (Arechavala-Lopez et al. 2013, 2014, 2018a,b, Dempster et al. 2018). External characteristics (e.g. colour patterns, fin erosion, scale features), coupled with morphometry (e.g. body and otolith shape, condition index), have proven to be highly accurate and easily manageable tools for discriminating the origins of gilthead seabream *Sparus aurata* due to their simplicity in deployment, which does not require broader expert knowledge or specialisation (Arechavala-Lopez et al. 2013). However, morphological resemblance between populations can also be viewed as a proxy measure for similarities in habitat use (Olsson et al. 2007), suggesting that morphometry analysis is primarily sensitive in the detection of recent escape events before the convergence from the farmed to the wild morphotype 'sets' in the natural habitat (Šegvić-Bubić et al. 2014).

In conjunction with selective breeding programmes targeting desirable production traits (e.g. high growth rate, disease resistance), farming conditions aim to further foster growth rates in cultured fish through a constant food supply at high stocking densities. Different foraging behaviour of farmed gilthead seabream in comparison to their wild counterparts (Arabaci et al. 2010) has likely enhanced the pattern of divergence from the spindle/elongated shape to the more inherent bulkier/stocky body form in cultured fish.

Similarities in terms of diet preferences of wild and farmed fish can be seen between fish farm-impacted areas and farm facilities (Arechavala et al. 2012b, 2014, Abaad et al. 2016). Sea cages have aggregative effects on local fish populations, impacting their presence, abundance, residence times and diet composition (Sanchez-Jerez et al. 2011). In the case of seabream and European seabass *Dicentrarchus labrax* farming, introduction of pellets into the marine environment influences the biological condition and metabolic status of surrounding fish due to the year-round feed availability of this food source and the different nutrient content in relation to natural food resources (Tuya et al. 2006, Fernandez-Jover et al. 2008, 2011, Sanchez-Jerez et al. 2011).

Research on the ecological impacts of inshore and coastal finfish farms on the surrounding environment and biota is well documented, whereas there is limited information for semi-offshore locations where tuna are farmed. Differences in the surrounding wild fish assemblages around tuna and other finfish farms are mainly associated with the type of feed used and the environmental conditions at the locations of these facilities (Šegvić-Bubić et al. 2011c, Stagličić et al. 2017). Tuna farms require excessive quantities of feed in the form of fresh or defrosted baitfish, which greatly differ from pellets that are used at seabream and seabass farms, in terms of proximate composition, leachability, dimensions and settling velocities (Vita et al. 2004, Aguado-Giménez & García-García 2005, Vassallo et al. 2006, Fernandes et al. 2007, Šegvić-Bubić et al. 2011c), forming high-quality habitats that enhance the fitness of coastal fish assemblages (Stagličić et al. 2017).

The Sparidae are one of the most abundant families found year-round in wild fish assemblages aggregating around tuna sea cages in Croatia (Šegvić-Bubić et al. 2011c, Stagličić et al. 2017). They are also exploited and farmed commercially for human consumption, and fished recreationally (Basurco et al. 2011). We selected the gilthead seabream as a representative species for the assessment of morphological and physiological adaptations to conditions in the wild, as a farmed species and in wild aggregations around tuna farms. The aim of this study was to: (1) differentiate body shapes according to origin, (2) evaluate the potential for identifying farmed escaped gilthead seabream in the wild using geometric morphometric tools and (3) improve our understanding of the effects of tuna farms on the fitness of wild gilthead seabream residing around these farms by assessing condition, gonadosomatic index and sex change during the spawning period.

2. MATERIALS AND METHODS

2.1. Fish sampling

A total of 1438 gilthead seabream were acquired from 2 wild tuna farm-associated, 5 farmed and 7 wild (but not farm-associated) populations in the eastern Adriatic Sea. Sampling was performed during the spawning season of the species from October to December in 2 years: 2015 and 2016 (Table 1, Fig. 1). The origin of farmed fish varied according to the fingerling supplier. Farmed gilthead seabream comprised individuals of French, Italian and Croatian origin. Farm-impacted areas were represented by 2 tuna farms located off the eastern coast of the Adri-

Table 1. Morphological data of gilthead seabream collected during 2015 and 2016 in the eastern Adriatic Sea. Conv.: conventional, N: total number of individuals sampled in both years, TL: total length, Wt: total weight

Sampling location	Site abbr.	Origin	N	2015	2016	TL (cm)		Wt (g)	
						Range	Mean ± SD	Range	Mean ± SD
Brač tuna farm	AB	Farm-associated	188	77	111	16.9–47.9	27 ± 3.6	80.1–1250	293.6 ± 128.6
Kali tuna farm	AK	Farm-associated	237	127	110	21.9–32.1	25.7 ± 1.8	141–451	230.2 ± 54.5
French offspring conv.	FR	Farmed	150	75	75	21.5–34.2	29 ± 2.9	148–594	399.7 ± 101.8
Italian offspring conv.	FI	Farmed	98	48	50	19.9–28	24.5 ± 1.9	114–495	230 ± 63.7
Italian offspring organic	FT	Farmed	97	47	50	25.5–30.3	28 ± 0.9	230.5–440	351.5 ± 39.3
Croatian offspring conv.	FC	Farmed	99	49	50	23.8–35.3	28.5 ± 2.4	212–768	368.4 ± 107.6
Croatian offspring organic	FH	Farmed	59	29	30	26.4–33.2	28.5 ± 1.9	303–701	401 ± 107
Dubrovnik	WD	Wild	57	57	–	24.3–50.9	31.7 ± 6	227–1502	477.9 ± 279.8
Istria	WI	Wild	99	40	59	22.8–29	26 ± 1.5	98.9–374	268.2 ± 54.3
Novigrad Sea	WN	Wild	90	57	33	21.6–26.4	23.78 ± 1.2	147.6–258.1	196.7 ± 27.3
Trogir	WG	Wild	31	–	31	22.9–28.5	26.3 ± 1.4	179–291	236.3 ± 30.6
Unije	WU	Wild	51	51	–	23.9–29.8	26.7 ± 1.5	191.3–416.9	293.7 ± 53.5
Velebit Channel	WV	Wild	97	60	37	22.6–30.1	25.9 ± 1.5	148–432.8	258.7 ± 52.3
Tar Bay	WT	Wild	85	41	44	12.2–24.7	19.7 ± 2.6	23–195	105.8 ± 36.4

atic Sea, 1 off the southwest coast of the island of Brač (AB) and the other off the southeast coast of the island of Ugljan (AK). Both facilities are located in 2 major aquaculture zones where tuna cage infrastructures are deployed 2 km from other finfish installations. Tuna-farming activities have been taking place for over 10 yr at semi-offshore locations settled 300 m away from the coastline at a depth of 50 to 60 m. The annual farming capacity of the Brač farm is around 900 t, with a sea concession surface of approximately 70 100 m², while the aquaculture concession of the Ugljan farm covers 420 600 m² of sea surface and has an annual production capacity of 1400 t. The 7 wild fish sampling locations were selected as unimpacted areas without farming activities, where the minimum distance between sampling localities and fish-farming activities was at least 50 km, and were distributed along the entire eastern Adriatic coast, from the Istrian Peninsula in the north to Dubrovnik in the south.

Farm-associated specimens were sampled by fish hooks in the vicinity of sea cages (~10 m max from the sea cage) at a depth of 40–60 m, while wild specimens were primarily obtained from fishing landings of local seiners at each location. Farmed population origin information was obtained from the farmers through the traceability of juvenile production or purchase documents.

2.2. Geometric morphometrics

For the purpose of this study, a digital camera carrier was constructed to facilitate the acquisition of body shape characteristics and to reduce the effect of parallax on geometric morphometric data (Mullin & Taylor 2002). All procedures regarding photo sessions and the digitalisation process were performed by the same person to minimise measurement error that can result from different positioning of the specimen under the camera and variation caused by multiple operators.

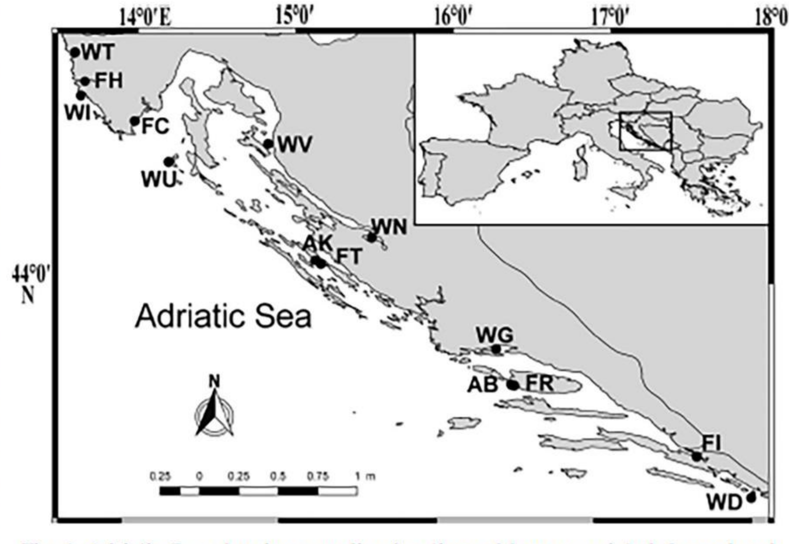


Fig. 1. Adriatic Sea, showing sampling locations of farm-associated, farmed and wild *Sparus aurata* populations along the eastern Adriatic coast. See Table 1 for abbreviations

Total length (cm) and weight (g) were recorded for each individual. Fish were placed at the centre of an expanded polystyrene block with 1 cm ruler gradation to ensure correct calibration in the following image data acquisition process. Body posture and fins were teased into a neutral position with needles of appropriate length to minimise the arching effect on the fish body. Lateral pictures of the left side of each sampled gilthead seabream were taken using a Canon EOS 600D digital camera.

Quantification and visualisation of gilthead seabream body shapes were attained by applying geometric morphometric methods (Bookstein 1991). A total of 18 anatomical landmarks were selected (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/q011p097_supp.pdf) to obtain the overall body shape of each specimen using TpsDig 2 software (Rohlf 2001). In the MorphoJ software package (Klingenberg 2011), generalized Procrustes superimposition was performed to separate size and shape components of form variation. Shape coordinates were computed from raw coordinates of configurations by scaling them to unit centroid size and by minimising differences in translation and rotation of all specimens until the sum of the squared distances (Procrustes distances) between the corresponding landmarks was minimised.

The amount of shape variation related to size was quantified by applying multivariate regression of the Procrustes coordinates on centroid size, with a permutation test using 10 000 runs in the MorphoJ software package. However, before the residuals of the regression could be used as 'size-free' variables in subsequent statistical shape analyses, a homogeneity of slopes test was performed in order to validate if a fit of a common regression line to all groups or to a common model using a pooled within-group covariance procedure could be obtained. By using the geomorph R package (Adams & Otarola-Castillo 2013), Procrustes ANOVA/regression for allometry with 1000 permutation runs was performed on the Procrustes-aligned coordinates obtained from MorphoJ.

Size composition differed among fish origins, and a small yet significant (4.2%, $p < 0.0001$) amount of shape variation was related to size. In addition, the homogeneity of slopes test recorded significant group allometries ($p < 0.001$), implying that a fit to a common regression line or to a common model for size correction of shape variables is not justified (Fig. S2). As one of the objectives of the study was to investigate how much body shapes differ between origins in the second and third age class (see means of population total lengths in Table 1), the effect of size (4.2%) on shape variables was not removed and as such was considered in further statistical analyses.

Canonical variate analysis (CVA) was performed in the MorphoJ software package to determine which shape features best distinguished the different origins of gilthead seabream. Pairwise comparisons of mean shapes from different origin populations were based on Procrustes distances, and a permutation test with 10 000 runs was performed to test the null hypothesis of no difference between samples. A Bonferroni correction for multiple tests for an experiment-wise error rate of 0.01 was used (Rice 1989). Moreover, Procrustes shape coordinates were also used to plot specimen ordination and to predict group affiliation using PAST 3.0 software (Hammer et al. 2001), applying the jack-knife cross-validation procedure.

2.3. Length–weight relationship, biometric indices and sex categorisation

Statistical analyses of the length–weight relationship (LWR), relative condition (*Kn*), gonadosomatic index (GSI) and multinomial logistic regression (MLR) were computed using R (R Core Team 2013) on a reduced dataset ($n = 1024$) including only individuals of 22 to 31 cm total length. The confined size range enabled exclusion of immature fish from the analysis considering that length–weight data are sensitive to slight differences in body shapes and growth stanzas. This is most evident in the early life stages of many fish species which have not yet obtained an adult body shape (Le Cren 1951, Safran 1992) and in older specimens whose body forms are often distorted due to unusually high proportions of fat (Froese 2006).

LWR and *Kn* represents 2 different, but interconnected, parameters in the analysis of length–weight data. LWR can provide morphological information due to the possible interpretation of the exponent b, whose deviation from 3.0 can suggest the direction and rate of change of form or condition, whereas *Kn* can be viewed as a short-term indicator of fish well-being since it is primarily influenced by food availability, body shape, gonad growth, sexual maturity and reproductive cycles (Hile 1936, Le Cren 1951, Policansky 1983, Pope & Kruse 2007). Analysis of LWR was based on the least-squares regression model ($y_i = \beta_0 + \beta_1 x_i + \varepsilon$), using the following equation:

$$\log_{10}(\mathrm{TW}) = a + b \times (\log_{10}\mathrm{TL}) \qquad (1)$$

where total weight (TW) corresponds to the dependent y_i and total length (TL) to the independent x_i variable, a (β_0) is the y-intercept, and b (β_1) is the slope of the line. Regression of $\log_{10}$TL × $\log_{10}$TW was performed to assess if different incremental weight gain and condition status existed between the observed origins of gilthead seabream in the eastern Adriatic Sea.

ANCOVA was used to compare regression lines of sampled length–weight data to test differences in slopes and intercepts. Building on the least-squares regression of $\log_{10}$TL × $\log_{10}$TW, the ANCOVA model is represented by the following equation:

$$\text{TW} = \beta_0 + \beta_1\text{TL} + \beta_2\text{Origin} + \beta_3\text{TL:Origin} \quad (2)$$

where TW is the response variable, β_0 is the y-intercept, TL is the covariate independent variable, Origin is the categorical independent variable that represents the effect on weight, and β_3TL:Origin is the interaction term. Tukey's post hoc test was applied to identify differences among origins in LWR, since differences in slopes can reveal changes in growth rate if the interaction term is significantly different from 0. In the case of no significant interaction, the regression lines are displayed in parallel fashion, indicating that the weight gain rate between observed origins is similar as individuals increase in length. Moreover, we wanted to investigate if there was a significant elevation difference between parallel regression lines through the analysis of intercepts, where discrepancies may indicate a better condition of one population over another at a given length (Pope & Kruse 2007). This was tested by replacing the interaction term with the following equation:

$$\text{TW} = \beta_0 + \beta_1\text{TL} + \beta_2\text{Origin} \quad (3)$$

The least-squares regression coefficients estimated from the LWR were also used to compare the condition status between farm-associated, farmed and wild specimens through Kn, since it compensates changes in body form or condition with increasing length (Le Cren 1951). Kn was calculated as:

$$Kn = \frac{TW}{W_c^{'}} \quad (4)$$

where $W_c^{'}$ is the predicted length-specific weight (a × TL^b) based on $\log_{10}$-transformed data. Specimens with higher or lower values of Kn are considered to be in better or worse condition, depending on their distance from the benchmark of 1.

Gonad weight per fish (GW) was measured to the nearest 0.01 g. Fish sex was determined macroscopically, and individuals were categorised as male, female or in the intersex phase (in cases when male and female parts of the gonads occurred together). Fish sampling during the spawning season allowed reliable sex categorisation of adults. The GSI was calculated on eviscerated fish (EW) as:

$$\text{GSI} = \frac{\text{GW}}{\text{EW}} \times 100 \quad (5)$$

The non-parametric Mann-Whitney U-test was used to test GSI variability of fish sampled in 2 consecutive years (2015 and 2016). Statistical differences of Kn and GSI among the observed origins were tested by ANOVA ($p < 0.05$) and Tukey's post hoc test. Assessment of the homogeneity of variances was performed through Levene's test.

Temperature has an effect on the speed of various reproductive processes (e.g. gonadal development, maturation, sex change etc.) and acts as a minimum/maximum threshold for fish spawning (Moretti et al. 1999). In order to explore sea surface temperature (SST) between the same period in different sampling years (2015 and 2016, October to December interval only), monthly averages and standard deviation of SST were calculated from daily averages of satellite SST. Daily averages of satellite SST interpolated for the Mediterranean Sea through an optimal interpolation algorithm were downloaded from the Copernicus Marine Environment Monitoring Service database (marine.copernicus.eu). The Consiglio Nazionale delle Richerce reprocessed the Pathfinder V5.2 (PFV52) AVHRR data over the period November 1981 to December 2017 to provide daily gap-free maps (L4) at the original PFV52 resolution at 0.0417° × 0.0417° (Pisano et al. 2016).

MLR was applied as a quantitative method for analysing categorical sex data and used for the likelihood evaluation of trends of sex change in protandrous hermaphrodite gilthead seabream for the observed length–weight interval. The application of MLR was carried out to predict the probabilities of the outcome of a categorical dependent sex variable, in which log odds of the outcomes were modelled as a linear combination of the continuous 'Total length' and the categorical 'Origin' predictor variable. The regression model for the multinomial sex response was developed by selecting the male sex category as the baseline for forming the odds of the remaining sex categories. Furthermore, the base level for the categorical 'Origin' variable was set to 'Wild', since our prediction of sex change in gilthead seabream (male → intersex phase → female) was based on the assumption that this occurs differently in farm-associated and farmed stocks in comparison to wild ones. In the present study, the MLR was expressed as:

$$\log \frac{\text{Prob } (y_i = j)}{\text{Prob } (y_i = \text{male sex})} = \beta_0 + \beta_1(\text{TL}) + \beta_2(\text{Origin}) + \beta_3(\text{TL:Origin}) \qquad (6)$$

where y_i is the response variable (sex), β_0 is the constant, $\beta_{1,2,3}$ are regression parameters that quantify the relationships between the predictor and response variables, and j is the observed sex category (intersex phase, female). Wald statistics were also computed to test if the regression coefficients of the predictors significantly contributed to the prediction of the response sex category outcome.

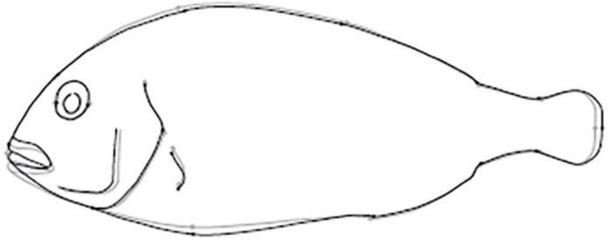
3. RESULTS

3.1. Geometric morphometrics

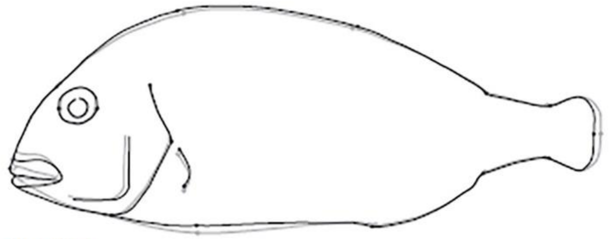
The sampled origins significantly differed in their average body shapes ($p < 0.0001$) for all 3 pairwise origin comparisons. Procrustes distances of farm-associated (FA) vs. farmed origin (FO) and FO vs. wild origin (WO) showed similar values (0.016 vs. 0.018) in contrast to the very weak distance observed for FA vs. WO fish (0.0066). The main body shape differences from the overall mean configuration were evident in the head and trunk region, dorsoventral body axes and caudal peduncle length (Fig. 2). FO fish were characterised by a stocky body form due to an extension of the abdomen region (Landmarks 10, 11, 12; see Fig. S1 for Landmark definitions) and contraction of the trunk (Landmarks 3, 4, 10, 11) and head area (Landmarks 1, 2, 3, 12, 13, 14). WO fish displayed a distinguishable spindle-like elongated body shape with the mouth positioned subterminally on a large head profile (extension of Landmarks 2, 3, 12, 13, 14). Body shape features of FA gilthead seabream appeared more similar to WO individuals, with head profiles slightly larger than in FO ones, but with a significantly bulkier body shape than in WO specimens.

The 2 canonical variates (eigenvalues 4.62 and 0.39) explained 92.14 and 7.86% of the total variance between landmark configurations in the overall sample. The jack-knifed discriminant analysis correctly classified 84.01% individuals to their origin (Table 2). The CVA ordination plot illustrated a pronounced discrimination in morphological features when comparing FO to FA and FO to WO (Fig. 3). The FO specimens had the highest correct classification rate (97.6%), demonstrating clear segregation from WO individuals, with minor overlap with FA specimens on the ordination plot (Table 2, Fig. 3). Misclassifications of FO fish were low, with 2% assigned to FA

A) Farm-associated



B) Farmed



C) Wild

Fig. 2. Warped outline drawings of mean shapes for gilthead seabream from different origins. The light grey outline represents the transformation from the overall mean shape, while the black outline represents the mean shape of each origin. Analysis of Procrustes distances showed that all 3 pairwise origin comparisons were significantly different ($p < 0.0001$). The shape differences were exaggerated 2-fold for better visualisation. The landmarks are defined in Fig. S1 in the Supplement

Table 2. Jack-knife classification score results (in counts and %) for the canonical variate analysis of the 3 origins of gilthead seabream from the eastern Adriatic Sea (FA: farm-associated, FO: farm origin, WO: wild origin). Overall, the jack-knifed discriminant analysis correctly classified 84.01% individuals to their origin

		FA	FO	WO	Total
Count	FA	329	11	85	425
	FO	10	491	2	503
	WO	120	2	388	510
	Total	504	475	459	1438
%	FA	77.40	2.60	20.00	100
	FO	2.00	97.60	0.40	100
	WO	23.52	0.40	76.08	100
	Total	35.05	33.03	31.92	100

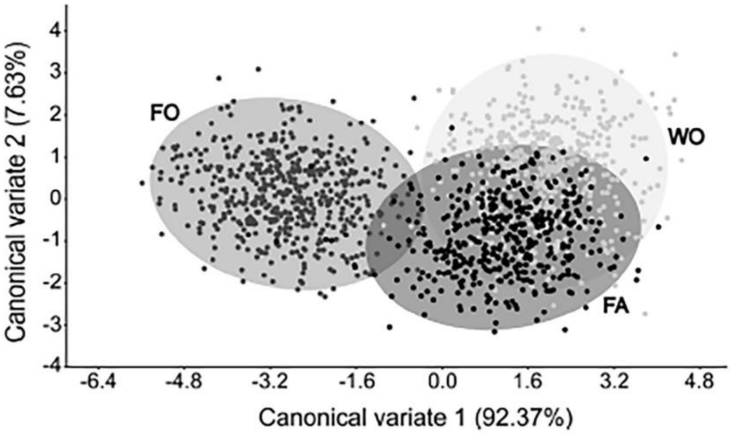


Fig. 3. Canonical variate ordination plot of sampled origins with 95% confidence ellipses of the scatter data points based on Procrustes distances. Farm-associated (FA) specimens are represented by black, farmed (FO) by dark grey and wild (WO) by light grey dots

origin, and 0.4% to WO. In FA and WO specimens, similar though higher misclassification assignments were recorded (22.6 and 23.92%, respectively), with considerable overlap of the scatter data by 95% confidence ellipses.

By exploring shape differences at the population level, the smallest Procrustes distance value (0.0046) was recorded among FA populations (AB–AK; see Table 1 for location definitions) and this was the only pair in the overall comparison matrix that displayed a non-significant p-value (see Table S1). Additional morphological resemblance was observed between several FA–WO populations (AK–WD, AK–WN, AB–WD, AB–WN) and among WO populations from the northern Adriatic (WU–WI, WI–WV). Other comparisons exhibited 1-fold higher distance values in contrast to those mentioned above (see Fig. S3, Table S1).

3.2. LWR and biometric indices

Regression of the transformed length–weight data of all origins showed a highly significant relationship ($p < 0.0001$, Table S2), where 84% of the variation in weight (r^2) was explained for the overall sample ($n = 1024$). ANCOVA displayed a significant TL:Origin interaction term ($F = 5.454$, $p < 0.01$), resulting in different weight gain rates between specimens of different origins with increasing length (Table 3). Post hoc Tukey's test revealed significant differences when comparing WO gilthead seabream with FA ($p < 0.01$) and FO specimens ($p < 0.05$), but no LWR slope difference was recorded among FA and FO fish ($p = 0.792$). As a result, a second model was fitted without the interaction term and compared with the previous one to investigate the discrepancy in the intercepts (see Section 2.3 for details). The additive model was more parsimonious since the fit of the model was not significantly affected by the removal of the interaction term ($F = 0.0068$, $p = 0.93$), thereby confirming the elevation difference between regression lines where FO gilthead seabream exhibited higher values of weight at given lengths in comparison with FA individuals with a similar incremental weight gain (Fig. 4).

Monthly mean SST for the eastern Adriatic Sea in October and in November during 2 consecutive sampling years was in accordance with the climatological mean monthly SST (Pastor et al. 2018), with a temperature deviation of 1 to 2°C from the mean values (Figs. S4 & S5). A Mann-Whitney *U*-test showed no significant difference ($U = 89570$, $p = 0.224$) of GSI values among sampling years. Prior to ANOVA, homoscedasticity of both tested variables, i.e. *Kn* and GSI, were confirmed by Levene's *F*-test. The ANOVA revealed significant differences in *Kn* between individuals of different origins ($F = 75.76$, $p < 0.01$), and all pairwise comparisons differed significantly (Tukey's post hoc test, $p < 0.01$). Moreover, GSI also differed significantly among fish origins ($F = 11.75$, $p < 0.01$), with significant interactions of FA vs. FO and FA vs. WO (Tukey s post hoc test, $p < 0.01$, Fig. 5), respectively.

Table 3. ANCOVA model and Tukey post hoc tests for length–weight relationship slope differences between farm-associated (FA), farmed (FO) and wild (WO) Adriatic gilthead seabream. TL: total length. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

ANCOVA	df	Type III SS	MS	*F*	Pr (>*F*)
logTL	1	2.523	2.524	2053.404	<0.000***
Origin	2	0.013	0.007	5.441	<0.004**
logTL:Origin	2	0.013	0.007	5.454	<0.004**
Residuals	1018	1.251			

Tukey post hoc test

Origin	Est. SD	SE	*z*	Pr (> |z|)
FO – FA	0.083	0.128	0.650	0.792
WO – FA	0.414	0.139	2.978	0.008**
WO – FO	0.331	0.119	2.760	0.016*

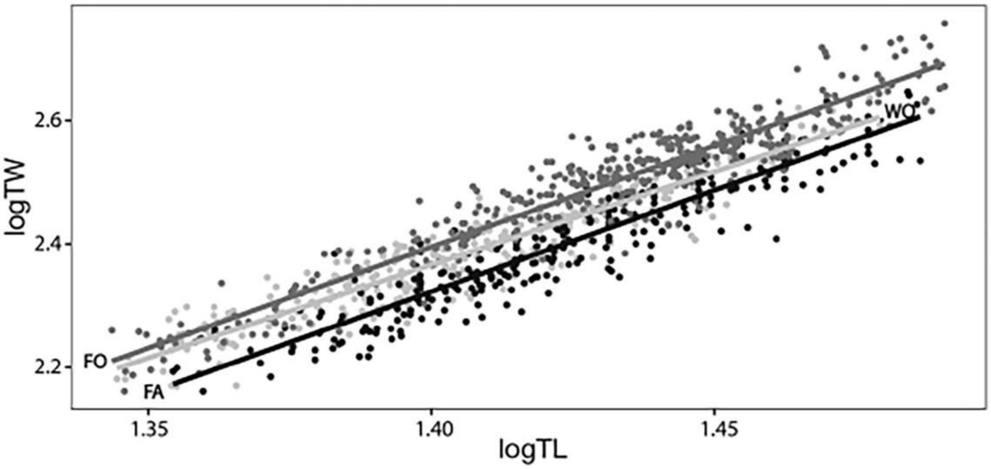


Fig. 4. Least-squares regression of logTL × logTW (where TL: total length and TW: total weight) of sampled gilthead seabream in the eastern Adriatic Sea. Farm-associated (FA) specimens are represented by black, farmed (FO) by dark grey and wild fish (WO) by light grey lines

3.3. Sex change

Overall, 71.3% of all specimens were male, 19.6% were in the intersex phase, and 9.04% were female (Table S3). FO populations exhibited the highest proportion of males (97.2%), while the intersex phase (27.4%) and female sex category (11.9%) were most prominent in fish of FA origin. Model parameters for MLR explained a significant amount of the original variability ($\chi^2 = 175.2$, $p < 0.01$). The transformed test statistic and the corresponding p-value provided sufficient evidence to conclude that the interaction term TL:Origin had an important effect on sex response categories (0.002424; $p < 0.01$). Sex change at given lengths depends on whether the gilthead seabream individuals were FO, FA or WO, where TL:Origin predicts whether or not individuals will undergo the change from male to female (Table 4). The odds ratio showed that as origin changes from WO to FA, individuals are more likely to undergo sex change with increasing length when examining parameter estimates from the male category to intersex phase ($\beta = 0.26$, Wald = 2.29, $p < 0.05$, Fig. 6). No significant interaction was recorded when observing the shift from WO to FO ($\beta = -0.06$, Wald = -0.18 $p > 0.05$), indicating that as length increases from 22 to 31 cm, FO specimens are unlikely to undergo the change to the intersex phase. Furthermore, a significant transition from male to female was noted with increasing length when observing the change between WO to FA ($\beta = 0.53$, Wald = 3.32, $p < 0.01$), but with no significant interaction recorded for the change between WO and FO ($\beta = 0.9$, Wald = 1.63, $p > 0.05$, Fig. 6).

4. DISCUSSION

In the present study, combined morphometric and biometric analyses were conducted to investigate phenotypic plasticity of gilthead seabream in rela-

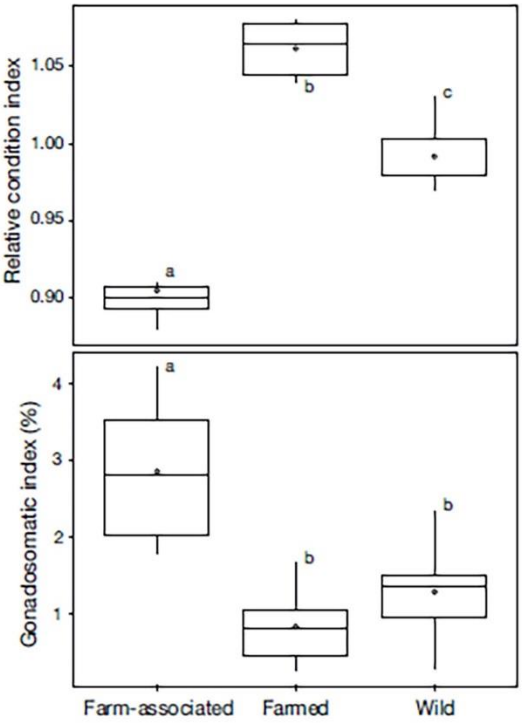


Fig. 5. Relative condition and gonadosomatic index of gilthead seabream. Same letters denote pairwise comparisons that are not statistically different

Table 4. Results of the multinomial logistic regression of sex change analysis. β: regression coefficient, Wald: β/SE, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

	β	SE	Wald	95% CI for odds ratio Lower	Odds ratio	Upper
Male vs. intersex phase						
Intercept	−3.88	1.95	−1.99*			
Length	0.11	0.08	1.52	0.97	1.21	1.29
FA	−6.97	3.03	−2.29*	0.00	0.00	0.36
FO	−2.24	9.61	−0.23	0.00	0.11	16.14
Length × FA	0.26	0.11	2.29*	1.04	1.30	1.63
Length × FO	−0.06	0.34	−0.18	0.48	0.94	1.83
Male vs. Female						
Intercept	−3.57	2.66	−1.33			
Length	0.07	0.10	0.69	0.87	1.07	1.31
FA	−14.38	4.31	−3.33***	0.00	0.00	0.00
FO	−29.20	16.81	−1.74	0.00	0.00	42.53
Length × FA	0.53	0.16	3.32***	1.25	1.71	2.34
Length × FO	0.90	0.55	1.63	0.83	2.47	7.31

tion to habitat use along the eastern Adriatic coast. This paper provides new insights into habitat effects on body morphology, LWR, condition, GSI and sex change of gilthead seabream originating from FA, FO and WO surroundings. For the first time, altered morphological and physiological traits were linked to semi-offshore tuna farm-impacted areas, with notably higher reproductive investment in FA populations in comparison to FO or WO populations.

The geometric morphometric analysis clearly demonstrated the divergence of FO body shape from FA and WO body form along the eastern Adriatic Sea. The highest level of morphological differentiation was observed in the head profile and anterior region of the fish body (see Fig. 2). CVA displayed FO gilthead seabream as fish with a stocky body form and smaller head profile with a superiorly positioned mouth, while WO individuals were distinguished by an elongated body shape and mouth positioned more subterminally on a larger head profile. FA individuals displayed a lower positioned nape and thicker belly compared to their wild counterparts and exhibited larger head profiles and a slimmer ventral body region. The inter-group classification for 3 origins showed an 84.01% correct classification score, with 2.6% misclassified assignments of FA and 0.4% of WO individuals to FO (see Table 2). Similar findings were also reported by Šegvić-Bubić et al. (2014) in Mali Ston Bay (Croatia), where 84% correct classification was observed for FO and WO individuals based on head, trunk and caudal peduncle measurements. Introgression of genes from cultured fish into the gene pool of wild counterparts and numerous reports of escape events have been reported within the eastern Adriatic (Šegvić-Bubić et al. 2011a, 2014, 2017, Glamuzina et al. 2014). Although reliable national statistics for any type of fishery landings are lacking, reports of gilthead sea bream landings have increased by 30.5% over the past 15 yr (Matić-Skoko et al. 2011). The influence of escape events on artisanal fisheries was also noted around the island of La Palma (Canary Islands), where an increment of aquaculture-based captures was reflected in the fisher association revenue in areas near the fish release point (Toledo-Guedes et al. 2014b). Studies on post-escape behaviour of farmed sea bream and seabass have shown dispersal movement patterns of escaped individuals, ranging from farm facilities to nearby farms, local fishing grounds and coastal habitats (Abecasis & Erzini 2008, Toledo-Guedes et al. 2009, Arechavala-Lopez et al. 2011, 2012b, Šegvić-Bubić et al. 2018). Therefore, the recorded misclassifications in the present study could indicate the presence of farmed gilthead seabream in wild populations, since findings along the eastern Adriatic coastline

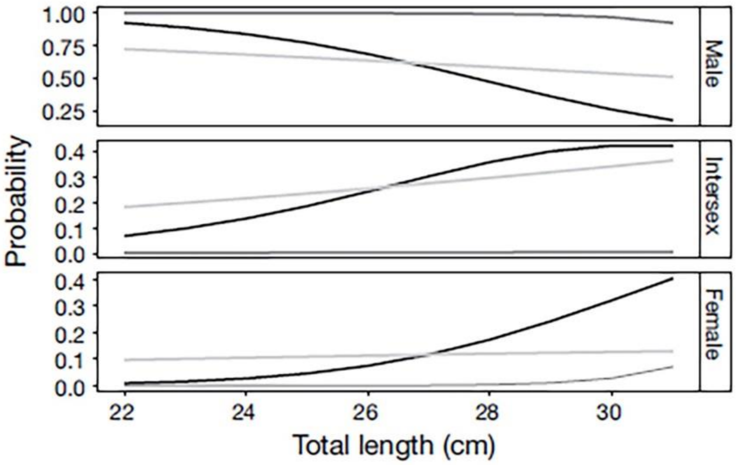


Fig. 6. Estimated multinomial regression model for sex data derived from interaction between 'Total length' and 'Origin' predictor variables. The predicted probabilities are plotted against total length of the gilthead seabream specimens by the 3 levels of origin for different categories of the sex outcome variable. Farm-associated specimens are represented by black, farmed by dark grey and wild by light grey line

revealed that escapees have contributed to the mix of wild and farmed genetic stocks, an increase of local populations, enhanced artisanal fisheries and adverse effects on the shellfish industry through predation (Šegvić-Bubić et al. 2011a,b, 2014, Glamuzina et al. 2014).

Gilthead seabream in the Mediterranean display a diversity of head profiles (i.e. normal, pointed, flattened and rounded) and body shapes (i.e. stocky, bulky, discoidal, elongated, stream-like, spindle-like) in wild and farmed populations (Loy et al. 1999, Grigorakis et al. 2002, Brown 2003, Russo et al. 2007, Hurtado-Rodriguez et al. 2010, Rogdakis et al. 2011, Arechavala-Lopez et al. 2012a, Šegvić-Bubić et al. 2014, Fragkoulis et al. 2017). Our results overlap with the findings of Arechavala-Lopez et al. (2012a) where a typical elongated-spindle like body shape and larger head profile was assigned to WO fish, while the stocky body form and smaller head profile was assigned to FO fish (see Figs. 2 & 3). Such adaptive plastic morphological responses, observed in the present fish origins, require certain environmental signals to trigger the development of the appropriate morphological feature (West-Eberhard 1989). Russo et al. (2007) revealed a significant correspondence between shape and feeding habit changes of gilthead seabream throughout its ontogeny as a result of adaptation to the availability and behaviour of different-sized prey. Rapid morphological changes were observed in the 10 to 40 mm size classes, primarily in the anterior region of the fish where shortening of the head and ventral positioning of the mouth was recorded, after which a stable stage was reached at 70 mm. Considering the high plastic response of jaws and head morphology to fish diet (Wringe et al. 2015), it can be stated that the observed differences in mouth positions of FO and WO gilthead seabream (see Fig. 2) reflect different ontogenetic diet shifts. For FO fish, early implementation of inert feed in the diet and 'from the surface to the bottom' feeding technology (see Russo et al. 2007) could explain the development of a superiorly positioned mouth. Rapid morphological divergence from the wild to the farmed morphotype has been observed in the first generation of wild-caught cod offspring (Wringe et al. 2015), indicating the importance of phenotypic plasticity as a mechanism of fishes that facilitates convergence of one morphotype into the other in concurrence with the given environmental conditions. Still, those typical morpho-characteristics attributed to farmed fish are not always a reliable tool for tracing fish origin, since the production of wild-like specimens can be achieved by applying mesocosm technologies in the form of hydrodynamism, environmental diversity and constant prey availability (Boglione et al. 2001, Shields 2001).

The applied geometric morphometric methodology in the present study enables morphological identification of FA fish sampled around tuna farms with some confidence, due to the partial shape overlaps with WO fish and similar misclassification results (see Figs. 2 & 3, Table 2). Interestingly, the average shapes of FA populations sampled around 2 different tuna farms (AB and AK) showed the highest resemblance, as opposed to other pairwise population comparisons (see Fig. 1, Table S1). It seems that the observed farms share similar environmental conditions, primarily in terms of trophic resources, which are responsible for triggering similar morphological adaptive responses in FA populations. Tuna farms are highly attractive habitats for surrounding wild fish whose aggregations of juvenile and adult stages persist year-round in large numbers, including gilthead seabreams (Šegvić-Bubić et al. 2011c, Stagličić et al. 2017). In addition to the fact that sea cages at fish farms add structure to the water column, analogous to large fish attraction devices (Dempster et al. 2011, Fernandez-Jover et al. 2011), the attraction of fish is also driven by large amounts of feed available at fish farms (Tuya et al. 2006, Fernandez-Jover et al. 2008, Sanchez-Jerez et al. 2011). Farmed bluefin tuna are fed a fat-rich diet composed of small pelagic species such as pilchard *Sardina pilchardus*, anchovy *Engraulis encrasicolus* and herring *Clupea harengus* (Katavić et al. 2003) to maximise body fat content for the demands of the sushi and sashimi markets (Mylonas et al. 2010). Although little is known about the estimated baitfish loss during feeding processes, losses of 4 to 23% were recorded for the southern bluefin tuna *Thunnus maccoyii* (Bruce 1997). This substantial portion of uneaten feed on tuna farms greatly increases feeding opportunities in the vicinity of cage infrastructures, enhancing foraging efficiency in aggregated wild fish. Furthermore, cage nets and supporting infrastructures offer thousands of square metres of surface for biofouling, upon which bivalves, the most common prey for wild seabream, constitute a large percentage of fouling communities (Fitridge et al. 2012). As opportunistic feeders, gilthead seabream will adapt their diet to the available food items in the habitat (Francescon et al. 1987, Pita et al. 2002, Arechavala-Lopez et al. 2012b), which has likely resulted in similar trophic plasticity and foraging performance between FA and WO populations residing in the vicinity of shellfish production areas (see Table S1).

Many fish species exhibit marked morphological changes during sexual maturation cycles (Good & Davidson 2016). Considering that the specimens in this study were sampled during the spawning period and that the chosen length range corresponds to the life stage when the majority of individuals start to consume somatic energy for various reproduction purposes (e.g. spawning, sex change), the effects of fitness indices on fish overall shape performance were noted. The highest GSI and the lowest *Kn*, as indicators of energy reserve status (Lloret et al. 2014), was observed in FA fish, where enlarged ovaries occupied most of the body cavity, restricting the space for food to be ingested. This in turn broadened the trunk area in contrast to their WO counterparts, whose slender ventral body region was facilitated by a substantially lower GSI. Despite the weight increment gains being similar to those in FA fish, the stockier body shape observed in FO fish was accompanied by high *Kn* and low GSI. Similar observations were also noted by Grigorakis et al. (2002) and Hurtado-Rodriguez et al. (2010), where a low GSI and increased condition factor were associated with a roundish body shape due to peritoneal and perivisceral fat accumulation in farmed gilthead seabream.

A similar SST variation pattern of 1 to 2°C was noted for the sample period (October and November, 2015 and 2016) and the region (Figs. S2 & S3), suggesting that the observed GSI variability among fish origins was mainly driven by the effect of tuna farming, although an effect of such small temperature variations among years and locations on gonadal development or sex change cannot be completely neglected. The higher reproductive potential of FA populations in comparison to FO and WO populations, seen through increased GSI and decreased *Kn* pattern in fish of FA origin, might be attributed to the fish-enriched diet in the vicinity of tuna farms. Such a diet satisfies the nutritional requirements for successful gametogenesis and viable production of eggs and larvae (Moretti et al. 1999, Izquierdo et al. 2001). It appears that FA populations have adopted the maximisation strategy of reproductive growth over energy store maximisation (Fernández-Palacios et al. 2011), which also facilitated the shape differentiation between the FA and WO fish to some extent.

The MLR for the observed length interval of 22–31 cm indicates that FA individuals are more prone to undergo sex change from male to female with increasing length compared to FO and WO specimens (see Table 4, Fig. 6). These results represent a deviation from the known sex change pattern of gilthead seabream, whose protandric hermaphrodite individuals are functional males in the first 2 yr, after which they become females once they reach ≥30 cm in length (Jardas 1996, Moretti et al. 1999). The secondary sexual developmental pathway in protandric species is strongly correlated with size/age, since larger females are able to produce more eggs while smaller males are able to produce enough sperm to fertilize both smaller and larger females (Ghiselin 1969, Warner 1988, Munday et al. 2006). The size effect concept in the theory of size-dependent sex allocation for sequential hermaphrodites (Klinkhamer et al. 1997, Leonard 2013) distinguishes (1) direct benefits of size per se, such as dominance in fishes living in groups, and (2) budget effects where greater energetic resources can be devoted to gamete production or to reproduction as opposed to growth or maintenance. The observed sex change results (see Table 4, Fig. 6) indicate that the budget effect of size is present in FA populations and that the lipid content of uneaten baitfish feed might support an increasingly female-biased sex allocation as individuals increase in size. The allocation of surplus energy to male functions beyond a certain point carries little reproductive return, since females have grown to a size at which they have sufficient reproductive success even at lower length classes. Furthermore, observed increased population density of gilthead seabream in farm-impacted areas (Šegvić-Bubić et al. 2011c, Stagličić et al. 2017) could also have further fostered sex change, since gender transition in hermaphrodites can occur in concert with changes in particular social/mating systems, habitat and population density (Munday et al. 2006, 2010, Avise 2011, Erisman et al. 2013). Thus, the plasticity of gonadal development and sex allocation, as a function of size/age, can be altered by the habitat and behavioural conditions in which gilthead seabream populations dwell.

In conclusion, this study provides insight into the complexity of gilthead seabream morphological and ecophysiological adaptations to different environmental settings, which could be attributed to the species reaction norms of phenotypic plasticity. In comparison to the classical or truss network measurement scheme, geometric morphometrics enabled visual interpretation of morphological discrimination between observed origin and, in conjugation with applied biometric indices, characterised components of the environment which these fish inhabited. Therefore, the establishment of databases on phenotypic and genotypic characteristics presents the next step in the effective management of cultured–wild fish interactions, and should be promoted on a larger regional scale in order to amend the detection sensitivity of morphological and genetic tools in future farmed-escapee identification in wild populations. Findings related to FA origin indicate that tuna farms form a unique ecological niche where wild aggregated populations have adapted their morphological and physiological states, particularly in terms of sex change and body shape. However, more research on their growth parameters, trophic structure and feeding strategies relative to reproduction are required to gain better insight into their phenotypic plasticity in comparison to their WO counterparts. This will not only contribute to the clarification of biological and ecological aspects of FA gilthead seabream, but will also help to resolve the role of tuna farms either as a population source or ecological trap for wild fish populations.

Data archive. The full dataset of farm-associated, farmed and wild gilthead seabream body shapes obtained from the eastern side of the Adriatic coast has been deposited into the GenoBase of the Institute of Oceanography and Fisheries (http://jadran.izor.hr/~tsegvic/aquapop/GenoBase.html) and is available from the corresponding author upon reasonable request.

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The following supplement accompanies the article

Morphological and ecophysiological adaptations of wild gilthead seabream *Sparus aurata* associated with tuna farms

Igor Talijančić, Tanja Šegvić-Bubić*, Iva Žužul, Tomislav Džoić, Jasna Maršić-Lučić, Leon Grubišić

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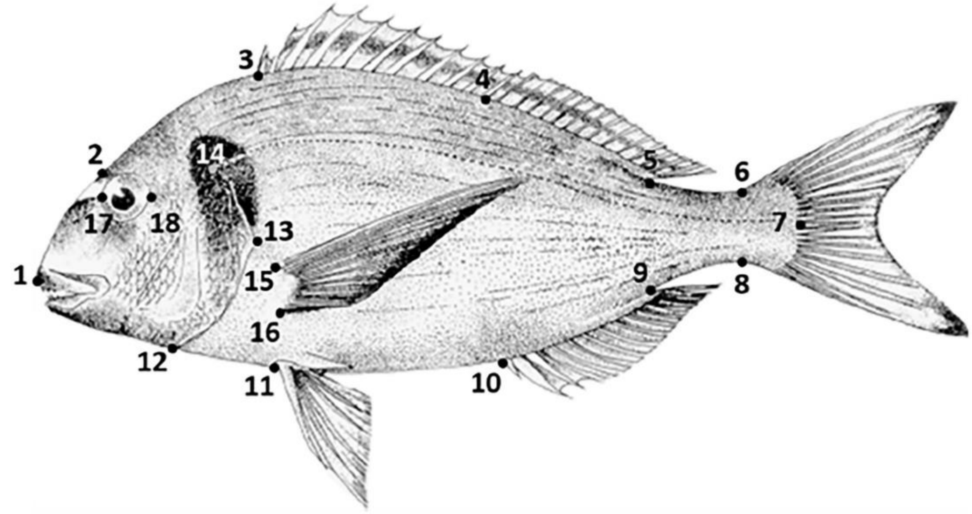

Fig. S1. Graphical representation of 18 landmark positions used to visualise the body shape of the sampled *Sparus aurata*: 1) anterior tip of snout at the upper jaw; 2) vertical point above the most anterior point in the eye; 3) anterior insertion of the dorsal fin; 4) last spiny ray of the dorsal fin; 5) posterior insertion of the dorsal fin; 6) dorsal point at the least depth of the caudal peduncle; 7) posterior body extremity; 8) ventral point at the least depth of the caudal peduncle; 9) posterior insertion of the anal fin; 10) anterior insertion of the anal fin; 11) insertion of the pelvic fin; 12) ventral tip of the insertion of the operculum on the lateral profile; 13) point of maximum extension of the operculum on the lateral profile; 14) anterior extremity of the lateral line on the head profile; 15) dorsal insertion of the pectoral fin; 16) ventral insertion of the pectoral fin; 17) the most anterior point in the eye; 18) the most posterior point in the eye

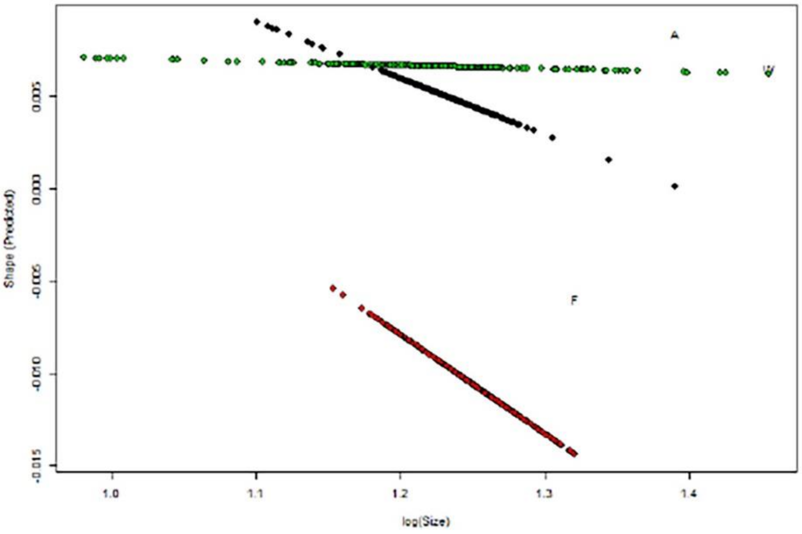

Fig. S2. Significant angular differences in the allometric slopes between sampled origins (W-wild, A-farm associated, F-farmed), where fish change shape as they grow in different ways, and vector length differences, where individuals from different origins change shape more as they grow, display heterogeneous patterns

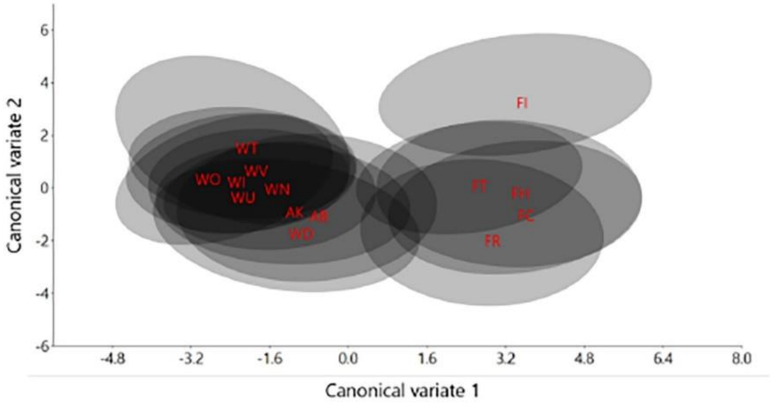

Fig. S3. Canonical variate (CV) ordination plot of sampled populations with displayed 95 % confidence ellipses. The nomenclature of the samples have been derived according to the: (a) origin of the fish (A – farm associated, F – farmed, W – Wild); (b) sampling location and farmed population origin with applied rearing technology (AB = Brač tuna farm; AB = Kali tuna farm; FR = French conventional; FI = Italian conventional; FT = Italian organic; FC = Croatian conventional; FH = Croatian organic; WD = Dubrovnik; WI = Istria; WN = Novigrad Sea; WO = Trogir; WU = Unije; WV = Velebit Channel; WT = Tar)

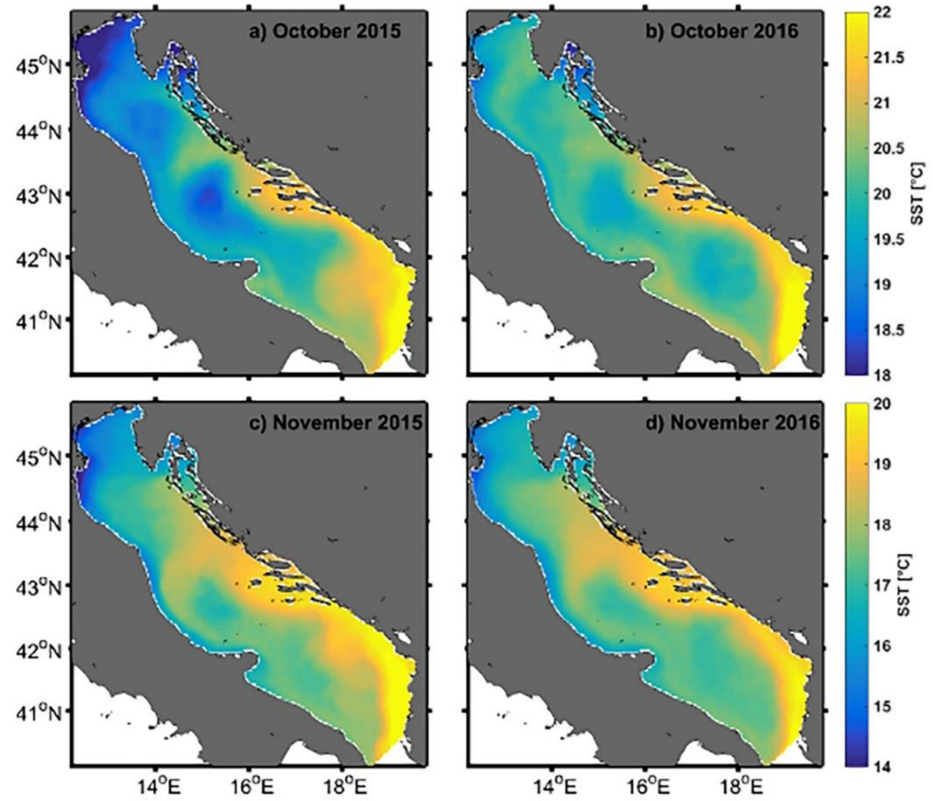

Fig. S4. Monthly mean SST obtained from reprocessed AVHRR satellite data during the sampled period in two consecutive years

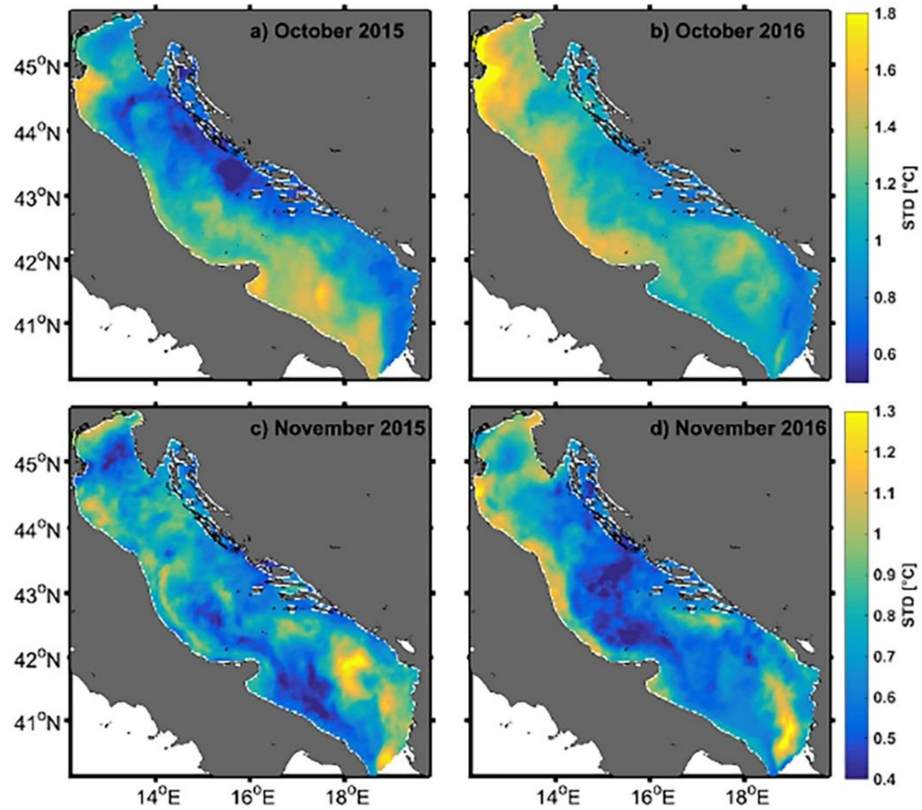

Fig. S5. SST standard deviation (STD) obtained from reprocessed AVHRR satellite data during the sampled period in two consecutive years

Table S1. Procrustes distances between mean body shapes of gilthead seabream from the sampled farm-associated, farmed and wild populations. Values in bold displayed pairwise comparisons of mean shapes between populations that are for one-fold smaller than the remaining pairwise comparisons

Population	AB	AK	FC	FH	FI	FR	FT	WD	WI	WN	WO	WT	WU
AK	**0.0046***	–	–	–	–	–	–	–	–	–	–	–	–
FC	0.0169	0.0201	–	–	–	–	–	–	–	–	–	–	–
FH	0.0186	0.0222	0.0122	–	–	–	–	–	–	–	–	–	–
FI	0.0218	0.0245	0.0211	0.0221	–	–	–	–	–	–	–	–	–
FR	0.0147	0.0185	**0.0085**	0.0115	0.0183	–	–	–	–	–	–	–	–
FT	0.0169	0.0198	0.0163	0.0130	0.0213	0.0144	–	–	–	–	–	–	–
WD	**0.0087**	**0.0086**	0.0217	0.0237	0.0229	0.0189	0.0214	–	–	–	–	–	–
WI	0.0138	0.0143	0.0233	0.0203	0.0264	0.0220	0.0181	0.0164	–	–	–	–	–
WN	**0.0083**	**0.0073**	0.0214	0.0226	0.0247	0.0197	0.0215	0.0121	0.0158	–	–	–	–
WO	0.0195	0.0156	0.0318	0.0352	0.0335	0.0316	0.0311	0.0185	0.0232	0.0186	–	–	–
WT	0.0185	0.0194	0.0220	0.0270	0.0253	0.0226	0.0283	0.0220	0.0235	0.0182	0.0277	–	–
WU	0.0121	0.0131	0.0219	0.0188	0.0269	0.0200	0.0160	0.0170	**0.0096**	0.0138	0.0230	0.0246	–
WV	0.0120	0.0109	0.0241	0.0230	0.0260	0.0230	0.0204	0.0143	**0.0079**	0.0122	0.0170	0.0215	0.0109

Bonferroni correction of *p* – value ($\alpha = 0.01/91 = 0.00011$). * A non-significant p value (0.0004) was recorded only for pairwise comparisons among farm-associated populations.

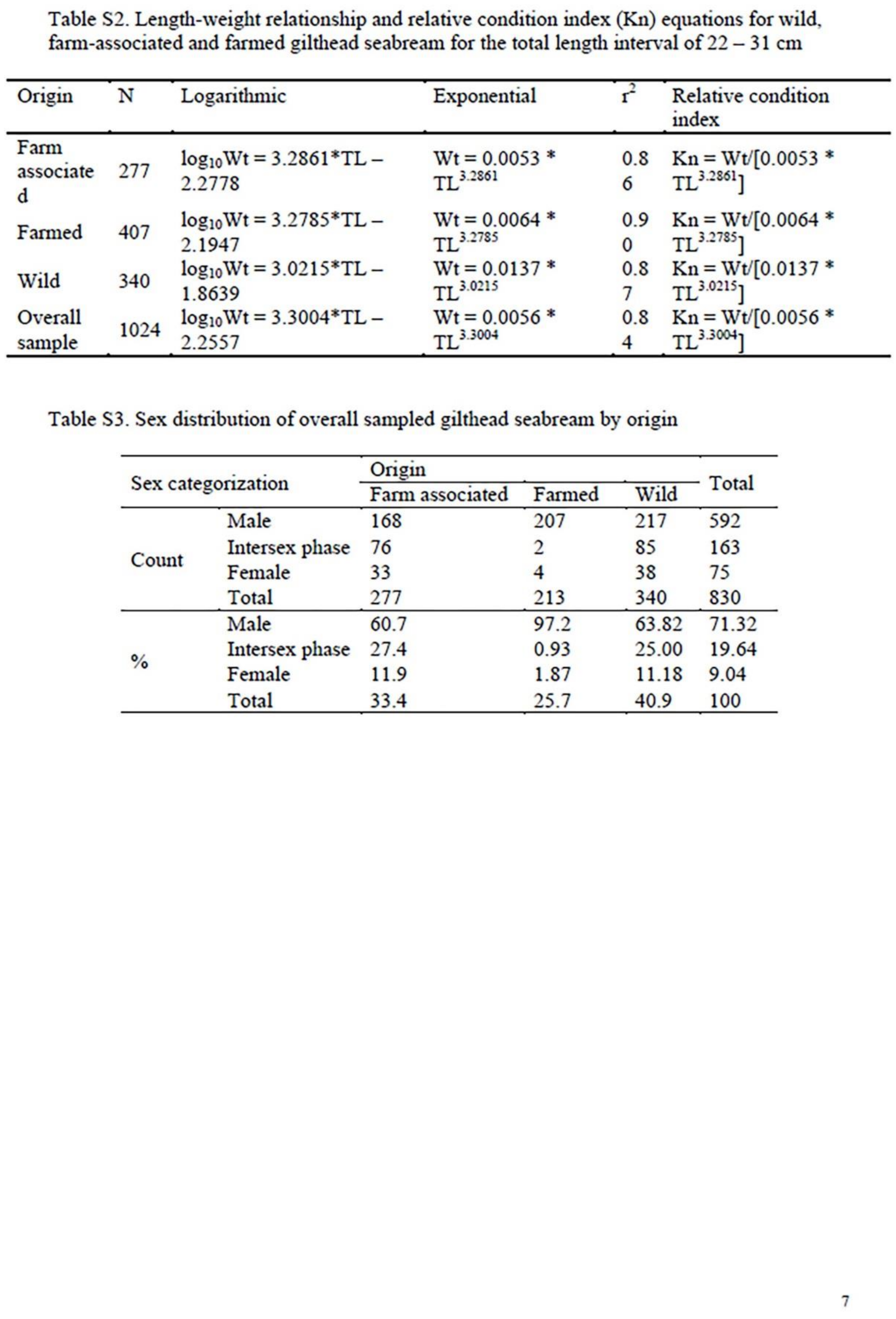

Table S2. Length-weight relationship and relative condition index (Kn) equations for wild, farm-associated and farmed gilthead seabream for the total length interval of 22 – 31 cm

Origin	N	Logarithmic	Exponential	r^2	Relative condition index
Farm associated	277	$\log_{10}Wt = 3.2861*TL - 2.2778$	$Wt = 0.0053 * TL^{3.2861}$	0.86	$Kn = Wt/[0.0053 * TL^{3.2861}]$
Farmed	407	$\log_{10}Wt = 3.2785*TL - 2.1947$	$Wt = 0.0064 * TL^{3.2785}$	0.90	$Kn = Wt/[0.0064 * TL^{3.2785}]$
Wild	340	$\log_{10}Wt = 3.0215*TL - 1.8639$	$Wt = 0.0137 * TL^{3.0215}$	0.87	$Kn = Wt/[0.0137 * TL^{3.0215}]$
Overall sample	1024	$\log_{10}Wt = 3.3004*TL - 2.2557$	$Wt = 0.0056 * TL^{3.3004}$	0.84	$Kn = Wt/[0.0056 * TL^{3.3004}]$

Table S3. Sex distribution of overall sampled gilthead seabream by origin

Sex categorization		Origin			Total
		Farm associated	Farmed	Wild	
Count	Male	168	207	217	592
	Intersex phase	76	2	85	163
	Female	33	4	38	75
	Total	277	213	340	830
%	Male	60.7	97.2	63.82	71.32
	Intersex phase	27.4	0.93	25.00	19.64
	Female	11.9	1.87	11.18	9.04
	Total	33.4	25.7	40.9	100

III. ČLANAK

frontiers
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ORIGINAL RESEARCH
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Plastic Responses of Gilthead Seabream *Sparus aurata* to Wild and Aquaculture Pressured Environments

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Fish farms, as artificial marine structures with a constant food supply, have a high capacity to attract surrounding wild fish. Different phenotypes of abundant gilthead seabream have been recorded in natural and aquaculture pressured environments in the eastern Adriatic Sea, where the influence of tuna farming on plastic traits associated with habitat use and foraging behaviour remains largely unknown. Several traits, such as body and otolith shape, external colouration, diet preference, and proximate tissue content, were analysed to examine habitat selection of the tuna farm-associated phenotype in comparison to wild and farmed phenotypes. Foraging behavioural shifts in prey selectivity, from hard-shelled bivalves towards soft textured baitfish feed, and local hydrodynamic conditions have initiated plastic responses in farm-associated seabream. Consequently, morphological traits connected with feeding and swimming performance and paler vs. vivid body colouration patterns differed between wild and farm-associated fish, highlighting the existence of resource polymorphism in gilthead seabream. While otolith shape proved to be a reliable phenotypic tracer in distinguishing farmed from wild and farm-associated fish, reduced sensitivity was found between individuals residing in the vicinity of tuna farms and wild ones. To fully understand the impact of fish farms on associated gilthead seabream and its plastic response with a distinctive morph outcome, the underlying molecular mechanisms involved in the regulation of alternative phenotypes needs to be investigated.

Keywords: aquaculture, gilthead seabream, phenotype, morphology, foraging, proximate composition, colour, otolith shape

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INTRODUCTION

Interaction and compatibility of aquaculture with the environment is one of the main debated issues linked to the sustainability of this food industry. Based on the expected trends in food consumption, development of farming technologies and their potential long-term ecological impacts on wild habitats, the European Union is anticipating to reach production of 109 million tonnes by 2030 (FAO, 2020). In the Mediterranean, mariculture has been accompanied by growing concerns about the potential impacts of escapees from cage installations on native populations through: (i) predation and competition for food; (ii) competition for space and breeding opportunities; (iii) the spread of parasites and diseases; and (iv) interbreeding with wild fish (Arechavala-Lopez et al., 2013b; Arechavala-Lopez et al., 2017). With an annual finfish production of 1.9 million tonnes (FAO, 2020) and over 21,000 finfish and tuna cages in the Mediterranean (Trujillo et al., 2012), farm leasehold areas also act as high-quality habitats that enhance the productivity of coastal fish assemblages (Sanchez-Jerez et al., 2011; Bayle-Sempere et al., 2013; Stagličić et al., 2017). Farming installations attract large fish aggregations by introducing structures and a constant food supply in form of uneaten feed into the pelagic environment, thereby enhancing the attractive effect on local populations by affecting their presence, abundance, residence time and diet (Dempster et al., 2002; Sanchez-Jerez et al., 2011; Šegvić-Bubić et al., 2011a; Stagličić et al., 2017). Due to eased foraging, wild fish assemblages have enrolled a new ecological role of mitigating the environmental impact of farms by consuming a large portion of the particulate waste generated from rearing activities (Vita et al., 2004; Ballester-Moltó et al., 2017). A recent world-wide assessment has documented up to 60 fish families with 170 species aggregating around sea-cage farms, where at least 17 species were recorded foraging on aquaculture feed (Sanchez-Jerez et al., 2011). A common feature emerging from Mediterranean studies is the consistent record of the family Sparidae around finfish and tuna farms, despite their discrepancies in attracted fish assemblages due to different site selection, i.e., coastal vs semi-offshore, and the proportion of uneaten and type of feed, i.e., pellets vs baitfish (Valle et al., 2007; Šegvić-Bubić et al., 2011a; Bacher et al., 2012; Stagličić et al., 2017).

Highly plastic responses to natural and/or aquaculture-induced environmental cues were observed in several studies of commercially important sparid species gilthead seabream, where significant phenotypic differences were noted between farmed fish and fish of wild origin (Rogdakis et al., 2011; Arechavala-Lopez et al., 2012; Šegvić-Bubić et al., 2014; Fragkoulis et al., 2016; Talijančić et al., 2019). Recently, the occurrence of a third phenotype was observed in the vicinity of tuna farms, where farm-associated individuals differed in morphological and ecophysiological traits compared to their wild counterparts (Talijančić et al., 2019), suggesting that plastic responses to novel environments tend to occur along phenotype components.

A significant increase of wild gilthead seabream has been documented in coastal areas of the eastern Adriatic Sea in recent years, mainly due to: (i) the introduction of domesticated individuals in wild populations through escape events (Šegvić-Bubić et al., 2011b, 2014; Glamuzina et al., 2014; Žužul et al., 2019); (ii) the ecological role of bluefin tuna (*Thunnus thynnus*) farms as populations sources for aggregated wild populations (Šegvić-Bubić et al., 2011a; Stagličić et al., 2017; Talijančić et al., 2019); and (iii) offspring spillover from tuna farm surroundings to natural nursery grounds of the species (Žužul et al., 2019). Despite the growing body of aquaculture impact research on phenotypic traits, it remains still relatively unknown how, and to what extent, the human activities associated with sea-cage farming influence the ecological attributes of seabream, such as habitat use, feeding habits, morphology, and life history traits. Thus, the aims of the present study were to reveal the factors that drive gilthead seabream adaptations within tuna farm-impacted areas, with reference to wild individuals in habitats with no cage farming, and to expand the base of distinguishable features between wild (WO), farm-associated (FA), and farmed (FO) fish. Since morphological structures require appropriate behaviours and physiological support for proper function, to meet the challenges presented by the environment (Norton et al., 1995), the specific objectives of this study were set to investigate: (i) how aspects of phenotype act to generate variability of body traits in wild and tuna farm-impacted habitats; (ii) applied foraging strategies of seabream in relation to length and disposable food resources; (iii) diet impact on muscle tissue quality composition, fatty acid profiles and skin colouration patterns and, finally, to explore (iv) otolith shape traits of WO, FO, and FA groups and their potential for fish origin classification.

MATERIALS AND METHODS

Study Area and Sample Collection

Fish from four wild, two farm-associated, and two farm populations were sampled along the eastern Adriatic Sea during the spawning season, October through December, in two consecutive years, 2015 and 2016 (**Supplementary Figure 1**). Farm-associated seabream were collected below tuna farms (AK, AB) located in two large aquaculture areas in Croatia, where the majority of seabream, seabass, and tuna farming activities take place. In collaboration with local commercial fishing vessels, wild individuals were sampled in four fishing areas (WD, WN, WV, and WI), where no escapees were expected due to the distance of at least 50 km from a fish farming area. Farmed seabream were obtained from two large commercial grow-out farms on the southwest coast of the island of Brač (FS1) and on the southeast coast of the Istrian peninsula (FS2).

A total of 947 adult gilthead seabreams with mean total lengths (TL ± SD) of 26.85 ± 2.6 cm, 26.35 ± 5.4 cm, and 27 ± 1.9 cm were collected for WO (n = 334), FA (n = 320), and FO origin (n = 293), respectively (**Table 1**). All WO and FA specimens were photographed on the left lateral side of the body using a Canon EOS 600D digital camera (n = 654). From the same sample, an 84% success rate for otolith extraction was achieved (WO, n = 317; FA, n = 232), while a 100% extraction rate was achieved for FO fish (n = 293). A subsample of 99 individuals was used for diet content analysis (WO, n = 42; FA, n = 57). Finally, examination of external colour appearance (n = 149) was performed only on digital images of individuals sampled for diet content analysis, with an additional 50 images sampled from FO fish.

Body Shape

Landmark-based geometric morphometrics (GM) was applied for body shape analysis on WO and FA dataset (n = 654), where a total of 38 points were digitised in tpsDig 2 (Rohlf, 2010), of these, 18 landmarks were collected as fixed, homologous points, and 19 semilandmarks as a curve outlining the head profile (**Supplementary Figure 2**). Semilandmarks were permitted to slide along their tangent directions using the Procrustes distance

TABLE 1 | Sampling location from the eastern Adriatic Sea, along with the origin and number of gilthead seabream *Sparus aurata* used in the present study.

Fish origin	Study area	Body shape analysis		Otolith shape analysis		Body colouration (BDC)/stomach content analysis (SCA)	
		2015	2016	2015	2016	BDC	SCA
Wild (WO)	WD—Dubrovnik	49	–	40	–	6	6
	WN—Novigrads Sea	57	33	55	33	22	22
	WV—Velebits channel	59	37	57	37	13	13
	WI—Istria	40	59	36	59	1	1
Total		205	129	188	129	42[a]	
Farm-associated (FA)	AB—Brač tuna farm	59	83	59	53	22	22
	AK—Kali tuna farm	95	83	65	55	35	35
Total		154	166	124	108	57[a]	
Farmed (FO)	FS1—Farm site 1	–	–	75	75	50	/
	FS2—Farm site 2	–	–	71	72		
Total				146	147	149	99

The number of individuals per population used in the analysis of body and otolith shape, body colouration and diet stomach content are also presented.

[a] *Same fish were used for external colour appearance and stomach content analysis.*

criterion. Configuration of points (x,y coordinates) were then subjected to a generalised Procrustes analysis (GPA) to retain shape variation by removing the effects of location, scale, and orientation with the gpagen function in the geomorph R package (Adams and Otarola-Castillo, 2013).

Prior to further analyses, allometric relationship of body shapes and log-transformed centroid size (CS) was studied using Procrustes coordinates. The function procD.allometry (geomorph R package) with group inclusion was used to quantify the relative amount of shape variation attributable to covariation with organism size. The homogeneity of slopes (HOS) test was performed to validate if the residuals of the multivariate regression (shape variables vs CS) could be used as size-free variables in subsequent statistical shape analyses. Furthermore, a multivariance comparison of variance within WO and FA origins was performed with vcvComp R package (Maître and Mitteroecker, 2019) in order to test interannual variability of body shapes before being pooled into a group.

Canonical variate analysis (CVA) was performed in MorphoJ software package (Klingenberg, 2011) to visualise body shape features that distinguish wild from farm-associated gilthead seabream. Procrustes shape coordinates were also extracted and used to predict group affiliation using PAST 3.0 software (Hammer and Harper, 2001) by applying the jack-knife cross-validation procedure.

Relative principal component analysis (relPCA) was performed with the vcvComp R package to identify body shape characters that exhibit maximal or minimal excess of variance between populations of WO and FA origin. Such an approach allowed for visualisation of morphological traits that exhibit a plastic response (i.e., higher variance) from those traits that show no response to environmental perturbations (i.e., lower variance). A likelihood ratio test of proportionality (ML) was used to test differences in variance patterns of populations, where a significant deviation from proportionality indicates the presence of divergent or stabilising selection regimes on body shape.

External Colour Appearance

A total of 149 seabream digital images were used for body colour pattern analysis of all three fish origins (**Table 1**). To obtain accurate colour data in red, green, and blue (RGB) colour space for subsequent variation analysis, the guideline of Stevens et al. (2007) was followed.

The comparison of colours and their patterns was performed with the colordistance (Weller and Westneat, 2019) and patternise (Van Belleghem et al., 2017) R packages. K-means clustering method was applied for the extraction of the dominant colour palette together with their pattern of spatial distribution. Due to the absence of distinct colour pattern boundaries in seabream, the number of clusters was defined manually through trial runs to distinguish the appropriate number of clusters that best assign pixels to a certain dominant colour pattern. The Colordistance R package was applied to quantitatively measure colour similarity among images by using the earth movers distance (EMD) metric to compare pixel colour clusters. Prior to examining whether the means of pairwise colour distance scores (CDS) differ between origins when performing the analysis of variance (ANOVA) comparison, the assumptions of homogeneity of variance and of normality were tested with the Levenes and Shapiro–Wilks tests. The Patternise R package was used to visualise variation in the extracted colour patterns along the outline of seabream body shape, where their alignment was performed with the intensity-based registration technique. For each cluster, the principal component analysis (PCA) and relative proportion of the colour pattern area was calculated in relation to the relative proportion of total body area, to differentiate and indicate possible overexpression of the extracted colour patterns in the observed origins. Statistical differences of pattern areas among the observed fish origins were tested by ANOVA and Tukey's *post hoc* test.

Foraging Patterns

A total 99 seabream from WO and FA origin were included in food choice modelling (**Table 1**). Specimens were weighed to nearest 0.1 g and their total lengths (TL) measured to nearest mm. Stomachs were eviscerated, weighed (wet weight), and preserved in 4% formaldehyde for content examination under a dissecting microscope with reflected light. Quantitative diet analysis was presented by applying three standard indices: the percentage frequency of occurrence (%F = the number of stomachs containing prey item/total number of non-empty stomachs × 100); the percentage numerical abundance (%N = the number of prey items of a given prey category in all non-empty stomachs/total number of prey items in all stomachs × 100); the gravimetric percentage (%W = the weight of prey items of a given prey category in all non-empty stomachs/total weight of food items in all stomachs × 100) (Hyslop, 1980). The contribution of each prey to the diet was assessed using the Index Relative Importance (% IRI) (Hacunda, 1981) based on three previous indices [IRI = (%N + %W) × %F], and expressed as a percentage [(IRI/ΣIRI) × 100].

Based on the prey category found in greatest volume, each fish was additionally categorised in respect to its primary food choice (PFC), i.e., preference to fish, bivalves or invertebrates (for details on PFC see Delany et al., 1999; **Supplementary Table S1**). Multinomial logistic regression (MLR) was applied as a quantitative method for analysing PFC and used for the likelihood evaluation of food choice trends in seabream in the 22–31 cm TL interval. To estimate response probabilities of a categorically dependent PFC variable, log odds of the outcomes were modelled as a linear combination of the continuous "Total length" and the categorical "Origin" predictor variable. The regression model was developed by selecting bivalves as the baseline for calculating the odds of the fish and invertebrate food categories, since bivalves are known to be the main prey of wild gilthead seabream in the Mediterranean Sea (Kara and Quignard, 2019). Furthermore, the baseline for the "Origin" variable was set to wild, due to the prediction that FA populations exhibit different foraging behaviour than wild ones. The baseline-category logit model was expressed as:

$$\log \frac{\text{Prob (Yi } = \text{j)}}{\text{Prob (Yi } = \text{bivalves)}} = \beta 0 + \beta 1(\text{TL}) + \beta 2(\text{Origin}) + \beta 3(\text{TL : Origin}),$$

where Yi is the response variable (food choice—PFC), β_0 is the constant, $\beta_{1,2,3}$ are regression parameters that quantifies the relationships between the predictor and response variables and *j* is the observed food category (fish, invertebrates). The likelihood ratio test was performed in the mlogit R package (Croissant, 2019) with the use of chi-square statistics for model fit evaluation. Wald statistics was computed to test if the regression coefficients significantly contributed to the prediction of the response food category outcome, whereas McFadden pseudo R^2 was used as a proxy for the evaluation of model fitting to the data, where values from 0.2 to 0.4 indicate excellent model fit (McFadden, 1987).

Body Proximate Composition and Fatty Acid Determination

To test the impact of diet preferences in relation to seabream origins, nutritional flesh quality evaluation was performed on 12 individuals, i.e., 4 fish per origin (WO, FO, and FA). Upon catch or harvest, fish were eviscerated and fillets were immediately frozen on dry ice before transferring to storage at -80°C. The fillets were homogenised with a laboratory homogeniser (Grindomix GM 200, Retsch, Haam, Germany) to create a uniform mince. AOAC standard methods were applied for proximate composition and fatty acid analyses, following the protocols of Pleadin et al. (2017).

Otolith Shape Analysis

Left sagittal otoliths were extracted from 842 individuals following the *open-the-hatch* method (Stevenson and Campana, 1992). After extraction, otoliths were mechanically cleaned and air dried before being digitised using a microscope Dino-Lite Premier. Black and white silhouettes were generated from the images in tpsDig 2 software while outline extraction was performed using the R package Momocs (Bonhomme et al., 2014). The silhouettes were converted into a list of x and y coordinates for each pixel around the contour of a given shape and transformed into quantitative descriptors using Elliptic Fourier analysis (EFA) through harmonic related equations. Due to the irregularities of the otolith shape, the outlines were smoothed to reduce the noise of digitisation by 100 smoothing iterations. Since round and symmetric configurations, such as otoliths, tend to have poor alignments, the superimposition of the extracted outlines was performed by a full generalised Procrustes alignment before EFA (for details see Claude, 2008), without normalising the descriptors. Harmonics were added until at least 99% of the otolith shape variance was reconstructed and Fourier descriptors were obtained for further analysis.

A PCA was performed on the matrix of descriptors to observe shape variation among origins. The principal components (PCs), expressing shape variability, were analysed with multivariance comparison of covariance (MANCOVA) to test the effects of origin and otolith size on seabream otolith shape. The allometric relationship test was performed with maximum or Feret otolith length due to absence of intersample differences in preservation, shrinkage and distortion in comparison to fish length measurements (Campana and Casselman, 1993). As recommended, a descriptor with a significant size effect needs to be removed to achieve an unbiased comparison of shape types (Rodgveller et al., 2017). Prior to further analyses, MANOVA for all three fish origins was performed with the Momocs R package to test for interannual variability of otolith shapes. Linear discriminant analysis (LDA) was conducted to discriminate predefined groups (WO, FO, and FA) of all analysed individuals, followed by otolith shape comparison among origins with a pairwise MANOVA test. The classification success of individuals into their sampled origin was estimated using a leave-one-out cross-validation procedure. Outline areas that most contributed to the difference between origins were identified by comparisons of mean shapes extracted from the descriptors and visualised with Thin-Plate Spline (TPS) analysis.

RESULTS

Body Shape

Size composition differed among populations, where a small yet significant (2%, $p < 0.001$) amount of shape variation was related to size. In addition, the HOS test recorded significant group allometries ($p < 0.001$), implying that a fit to a common regression line or to a common model for size correction of shape variables is not justified (**Supplementary Table S2**). Therefore, the effect of size on shape variables was not removed. The ML test displayed an absence of significant interannual variability of body shapes for WO ($p = 0.22$) and FA ($p = 0.29$) samples, allowing populations sampled in different years to be pooled into a single origin group.

The WO and FA origins differed in their average body shapes ($p < 0.0001$), but with small pair-wise Procrustes distance (0.0063). The main body shape differences were evident in head profile and the posterior part of the body, where FA fish were characterised with a slightly upwards position of the mouth on a smaller head profile and a fusiform body shape in comparison to their wild counterparts (**Supplementary Figures 3, 4**). The jack-knifed discriminant analysis correctly classified 85.17% individuals to their origins, where FA specimens exhibited a classification rate of 86.56%, as opposed to wild individuals that displayed 83.83% correct classification (**Supplementary Table S3**).

The multivariate comparison of variance was based on the first five PCs of the full Procrustes data, in order to avoid collinearities and to obtain a sufficient excess of cases over variables in the further analysis. The first three principal coordinates together accounted for 81.4% of the total variance (**Figure 1**). Differences in variance-covariance patterns between FA and WO populations were demonstrated by comparing farm-associated population (AB) with southern and norther wild populations (WD and WV), though other pairs of WO and FA population gave similar results. The ML test indicated that the covariance matrices of AB and WD ($p = 0.038$), and of AB and WV ($p = 0.002$), deviated significantly from proportionality. RelPCA analysis showed that the various features deviate in their variational properties across populations (**Figure 2A**) where the first relative PC was roughly twice as high in AB than in WD (first relative eigenvalue of 1.92) and WV (first relative eigenvalue of 2.13), whereas the variance of the last relative PC in AB was only the half of that in WD and WV (last eigenvalues of 0.53). The shape features captured by relative PC1 were head shape and the anatomical position of eyes, mouth and pectoral fins (**Figure 2B**). These morphological traits exhibited maximal excess of variance in FA populations, relative to WO or, in other words, they were maximally canalised in the wild individuals.

TABLE 2 | Classification of within- and between-origin silvery-grey colour similarity in gilthead seabream.

	Within-origin similarity (%)		
	Farm-associated	**Farmed**	**Wild**
Highly similar	14.22	71.67	11.30
Similar	58.08	24.82	61.71
Dissimilar	0.97	0.00	0.12
	Between-origin similarity (%)		
	Farm-associated vs farmed	**Wild vs farmed**	**Farm-associated vs wild**
Highly similar	9.24	1.95	7.39
Similar	36.86	24.33	59.03
Dissimilar	3.72	9.38	1.15

Analyses were based on predefined cut-off colour distance scores derived from the EMD distance colour matrix of sampled images: highly similar <0.005; similar $0.005 > x < 0.01$; dissimilar >0.02 (see ***Supplementary Figure 6****).*

External Colour Appearance

From the dataset including all three fish origins (WO, FA, and FO), body colours were k-means clustered into four clusters, ranging from dark to light silvery-grey hues (**Figure 3**). The highest distribution uniformity of pairwise CDS was noted within the FO origin, whereas the other two origins demonstrated much wider distributions (**Supplementary Figure 5**). Levene's and Shapiro–Wilks test indicated that the assumptions of homogeneity of variance ($F = 125.06$, $p < 0.01$) and normality (all origins—$p < 0.01$) were not met and, thus, Welch ANOVA for unequal variances was performed (**Supplementary Table S4**), together with the Games-Howell *post hoc* test. A statistically significant difference between origins was recorded ($F = 895.85$, $p < 0.01$) where approximately 42% ($\omega^2 = 0.419$) of the total CDS variance was attributable to origin (three levels). The *post hoc* test revealed that the pairwise comparisons WO vs FO and FA vs FO exhibited significant difference ($p < 0.01$), whereas the WO vs FA comparison displayed a non-significant difference ($p = 0.96$). Furthermore, EMD distance colour analysis recorded high within-origin similarity in FO, where 71.67% of individuals displayed the same grey hues in contrast to their wild conspecifics, which showed only 11.3% resemblance in the extracted colours. In the case of grey colour resemblance between origins, FA and FO showed a 9.24% similarity, as opposed to WO and FO, which displayed only 1.95% (**Table 2**).

The heatmap visualisation of colour patterns expression showed that FO had the dullest colouration, exhibiting darker grey colouration (lowest RGB values) in relation to the brighter silvery-grey colouration of the WO and FA populations. The first and fourth cluster of FO displayed the highest proportion rates, with prominent pattern distributions on the posterior part of the operculum, head profile, nape and the snout, dorsal and ventral body area, and caudal fin (**Figure 3**). The high proportion of the light grey colour cluster (IV) on the ventral body area more strongly emphasised the contrast with the dark grey dorsal areas, further enhancing the dull colouration of FO. The PCA analysis displayed a segregation between darker FO fish from WO and FA individuals (**Figure 4**). Similar RGB values and colour pattern coverage (%) areas were noted between WO and FA

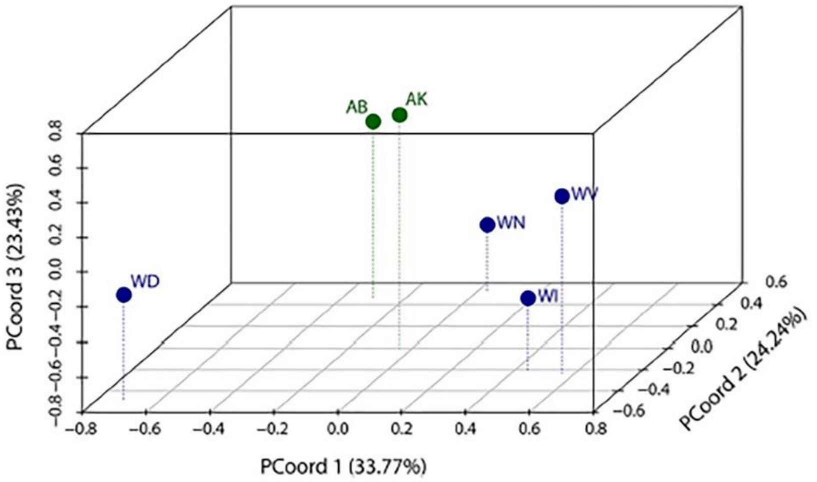

FIGURE 1 | Scatterplot of the first three principal coordinates (PCoord) of the relative principal component analysis. Populations living in wild are shown in dark blue whereas farm-associated populations are displayed in green colour.

populations, indicating that they do not differ substantially in the extracted silvery grey colouration. A non-significant difference in the pattern areas of the colour clusters was observed only for the fourth cluster (p = 0.144; **Supplementary Table S5**). Furthermore, post hoc Tukey's test on the first and second cluster revealed significant differences in the comparison of FO with WO and FA ($p < 0.01$), whereas for the third cluster significant differences were noted when comparing FA with FO and WO ($p < 0.01$).

Foraging Patterns

The quantitative gut content analysis is presented in the overall list of determinable taxa (**Supplementary Table S6**). The MLR chi-square test revealed a statistical significance in the overall model fit (log-likelihood = −69.698, χ^2 = 52.9, $p < 0.001$, McFadden R^2 = 0.27). A significant interaction was found between the variables Total length and Origin (β = −0.94, Wald = −1.97, $p < 0.05$), revealing that FA individuals were less likely to choose fish over bivalves as food than their wild counterparts, who showed a higher probability of choosing fish over bivalves as they increased in size (**Table 3** and **Figure 5**).

Body Proximate Composition and Fatty Acid Profiles

The chemical and mineral compositions of gilthead seabream muscle tissue are shown in **Supplementary Table S7**. The FO group had a higher fat content in comparison to WO and FA groups, whereas protein component and mineral composition of fillets were in a similar range for all observed origins. Fatty acid composition showed substantial variation among fish origins (**Supplementary Table S7**). While the most abundant fatty acids, oleic and linoleic acids, were substantially more abundant in the FO group, the opposite was noted for palmitic and stearic acid, which were more abundant in the WO and FA groups. The highest proportions of monounsaturated (MUFAs), polyunsaturated fatty acids (PUFAs), total n-3 and n-6 fatty acids, and EPA/DHA ratio were recorded in the FO group, whereas the highest n-3/n-6 ratio was observed in FA fish.

Otolith Shape Analysis

The EFA analysis was able to describe otolith shape by applying 14 elliptical harmonics, extracting 56 individual descriptors. According to MANCOVA, which tested the allometric relationship of otolith length and shape, no significant differences were observed (p = 0.6) and, therefore, all Fourier descriptors were included in the subsequent analyses. The MANOVA displayed an absence of interannual variability of otolith shapes within WO (p = 0.28), FA (p = 0.31), and FO (p = 0.54) samples, allowing both years to be pooled into a single group for each origin. The PCA showed that the first two components account for 62.3% of the total variance. The first axis (PC1, 47.8%) indicated an elongation or suppression of the excisure area, while the variation of the second axis (PC2, 14.5%) was linked to general shape changes associated with a rounding of the otolith, particularly in the posterior region (**Figure 6**). Variability within the FO sample was considerable, due to the notched anterior region which emphasised the rostrum and antirostrum areas (**Supplementary Figure 7**), in comparison with the WO and FA shapes, which displayed an angled to peaked anterior region with wide excisura (without or with a shallow notch), and a short, broad and pointed rostrum.

When attempting to classify otoliths into a group, LDA recorded an overall correct classification rate of 65.8%. Otoliths from FO showed a clear segregation from the WO and FA groups with 87.37% correct classification, as opposed to a 70.98% correct classification rate for WO (**Table 4**). Due to the overlapping shape pattern among FA and WO, a high misclassification rate of 54.3% was recorded for the FA otoliths classified as WO, and 14.2% as

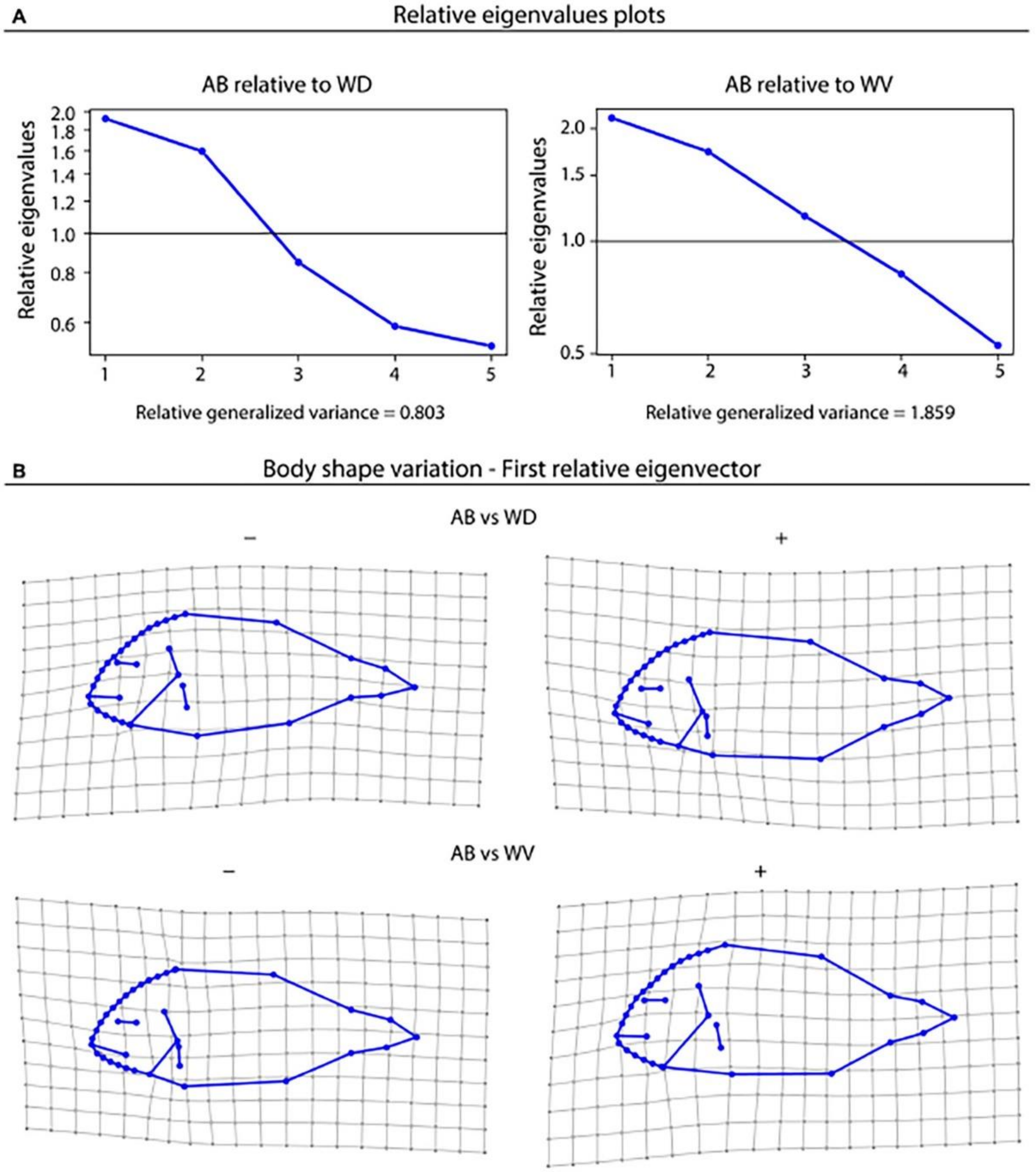

FIGURE 2 | (A) Eigenanalysis of the population comparisons of AB vs WD and AB vs WV; **(B)** Thin-plate spline deformation grids visualisation of the shape pattern that corresponds to the first relative PC, displaying the maximal excess of variance in AB relative to WD and WV.

FO. Pairwise MANOVA indicated a significant difference among all origins ($p < 0.001$). The mean morphological differentiations were related to the rostrum, antirostrum, ventral edge and postrostrum region (**Figure 7**). The FO fish displayed a significant indentation of the excisura area in relation to WO and FA, which mostly differed on the ventral edge and postrostrum part of the otolith.

DISCUSSION

Phenotypic plasticity can be seen as the ability of individual to produce multiple phenotypes under different environmental conditions (Skúlason et al., 2019). As one of its aspects, trophic or resource-based polymorphism presents the occurrence of intraspecific morphs that show differences in feeding biology or

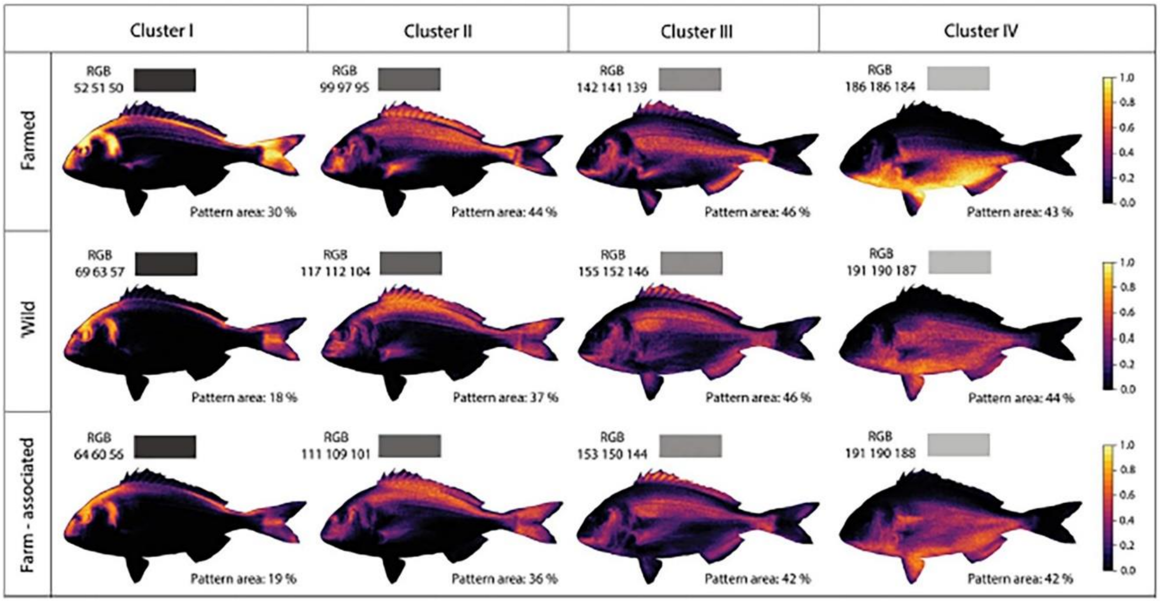

FIGURE 3 | Image registration and k-means clustering of the colour patterns of gilthead seabream. For the wild, farmed, and farm-associated origins (rows) colours were k-means clustered into four groups (columns), ranging from dark to brighter silvery—grey colour hues with displayed average RBG values and coverage (%) of extracted colour pattern area along the body surface area.

habitat use (Smith and Skúlason, 1996). In particular, habitat diversity and resource niches play an important role in fostering the appearance of phenotypic variants within a population of a single species; these are known as morphs and may differ in morphology, colour, behaviour, or life history traits (Skúlason and Smith, 1995; Smith and Skúlason, 1996). The occurrence of several morphologically and genetically distinct gilthead seabream populations have been recognised in the eastern Adriatic Sea, and classified as being of farmed, wild or farm-associated origin (Šegvić-Bubić et al., 2014; Stagličić et al., 2017; Talijančić et al., 2019; Žužul et al., 2019). In the present study, a multidisciplinary approach was used to explore the impact of aquaculture altered habitats, such as tuna farm leasehold areas, on the occurrence of farm-associated fish phenotypes, through analyses of body and otoliths shape, external colouration and dietary preferences.

Fish Body Shape Characteristics and Its Variation According to Habitat and Trophic Resources

The body shape dataset analysed here described the farm-associated gilthead seabream as a fish with a slightly upwardly positioned mouth on a smaller head profile, where a blunter snout was noted due to diminished head curvature, and with a more fusiform body shape tapering towards the tail in comparison to their wild counterparts (see **Supplementary Figure 3**). These differences between fish origins may appear minor, though the classification test revealed a high number of correctly assigned individuals (85%), with only 13% of farm-associated fish of misclassified as being of wild origin (see **Supplementary Table S3** and **Supplementary Figure 4**). Inclusion of head curvature shaped by 19 semilandmarks into the GM analysis generated an even higher correct classification score, in contrast to a study where only fixed landmarks were used on the same dataset, achieving a total correct classification score of 77% (Talijančić et al., 2019). Such an approach enabled better resolution of morphological trait variabilities within and among fish origins.

Namely, a two-fold higher variability of head shape, orientation of mouth and positions of eyes and paired fins was found within farm-associated populations than in wild populations (**Figure 2B**). This discrepancy in trait variability can be linked with resource variation of these environments, where the type, availability and abundance of resources all influence the overall fish morphology. In comparison to natural habitats, tuna farms provide additional structural complexity in the water column through sea cage infrastructures, food availability via baitfish loss and high hydrodynamic complexity, due to site selection criteria that require localities with high current velocities (Šegvić-Bubić et al., 2011a; Stagličić et al., 2017). The present study noted differences in dietary indexes (see **Supplementary Table S6**) where farm-associated individuals consumed fish prey (% IRI = 92) in contrast to the bivalve-based diet of wild fish (% IRI = 75), confirming the role of baitfish feed as the main trophic resource for wild aggregated fish populations (Fernandez-Jover et al., 2020). Thus, dietary and behavioural shifts, as reflected in the foraging position and type

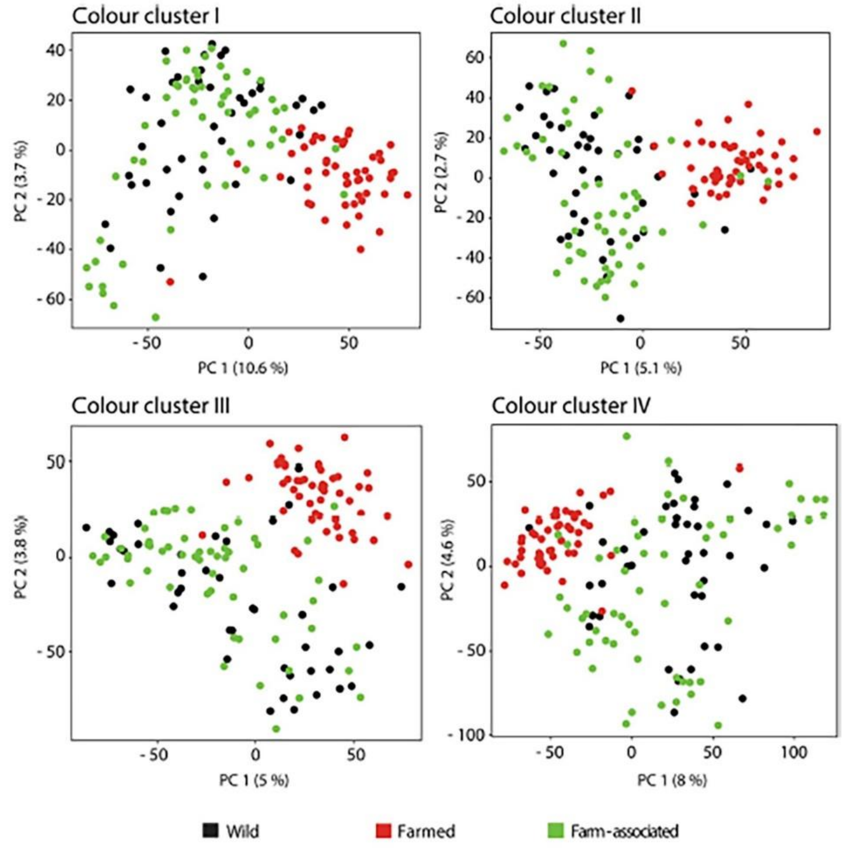

FIGURE 4 | Principal component analysis of the pixel matrices obtained for extracted clusters of silvery—grey colour hues.

of prey consumed, were likely the prevailing factors responsible for initiating morphological plastic responses.

Farm-associated seabream exhibited a more fusiform body shape overall, due to the deeper/wider anterior body, tapering towards the tail region (see **Supplementary Figure 3**). This observed body shape enables better resource exploration and reflects morphological adaptation that provide an advantage under high hydrodynamic water conditions (Bracciali et al., 2016). High variation in fin position indicates the adjustment in locomotor repertoires in concordance with foraging behaviour where fish was the preferred dietary category. While durophagous wild seabream feeds mainly by grasing on prey in natural environments (Kara and Quignard, 2019), non-evasive and free-falling baitfish feed induces greater use of the paired fins to perform precise manoeuvres in any plane to catch the preferred prey (Webb, 1984). The correspondence of shape changes with the general pattern of dietary shifts, the transition from smaller and softer to larger and harder prey, has been noted previously in gilthead seabream, particularly in the head region and body profile (Russo et al., 2007, 2014). It can be speculated that adaptation of gilthead seabream to capture baitfish from the water column also triggered the development of a more upturned mouth, where the increased head shape variability reflects less frequent use of crushing force due to a monotonous, soft diet based on fish. A smaller head profile with blunter snout was also observed in farm-associated salema *Sarpa salpa* and bogue *Boops boops* residing around finfish sea cages (Abaad et al., 2016). Regardless of

TABLE 3 | Estimated parameters in baseline-category logit model for gilthead seabream dietary preference, based on the quantitative total length indicator variable for size.

			Origin	**Interaction**
Logit	**Intercept**	**TL**	**FA**	**TL × FA**
log (π_F/π_B)	−8.93	−0.31 (0.22)[a]	29.31 (13.35)	−0.94 (0.47)
log (π_I/π_B)	1.45	−0.11 (0.26)	20.96 (14.24)	−0.65 (0.52)
Wald statistics				
log (π_F/π_B)	−1.54 (0.12)[b]	1.40 (0.16)	2.19 (0.02)	−1.97 (0.04)
log (π_I/π_B)	0.22 (0.82)	−0.41 (0.67)	1.47 (0.14)	−1.26 (0.20)

In parentheses: a—Standard error values, b—p values for Wald statistics. Subscripts: F—fish, B—bivalve, I—invertebrate.

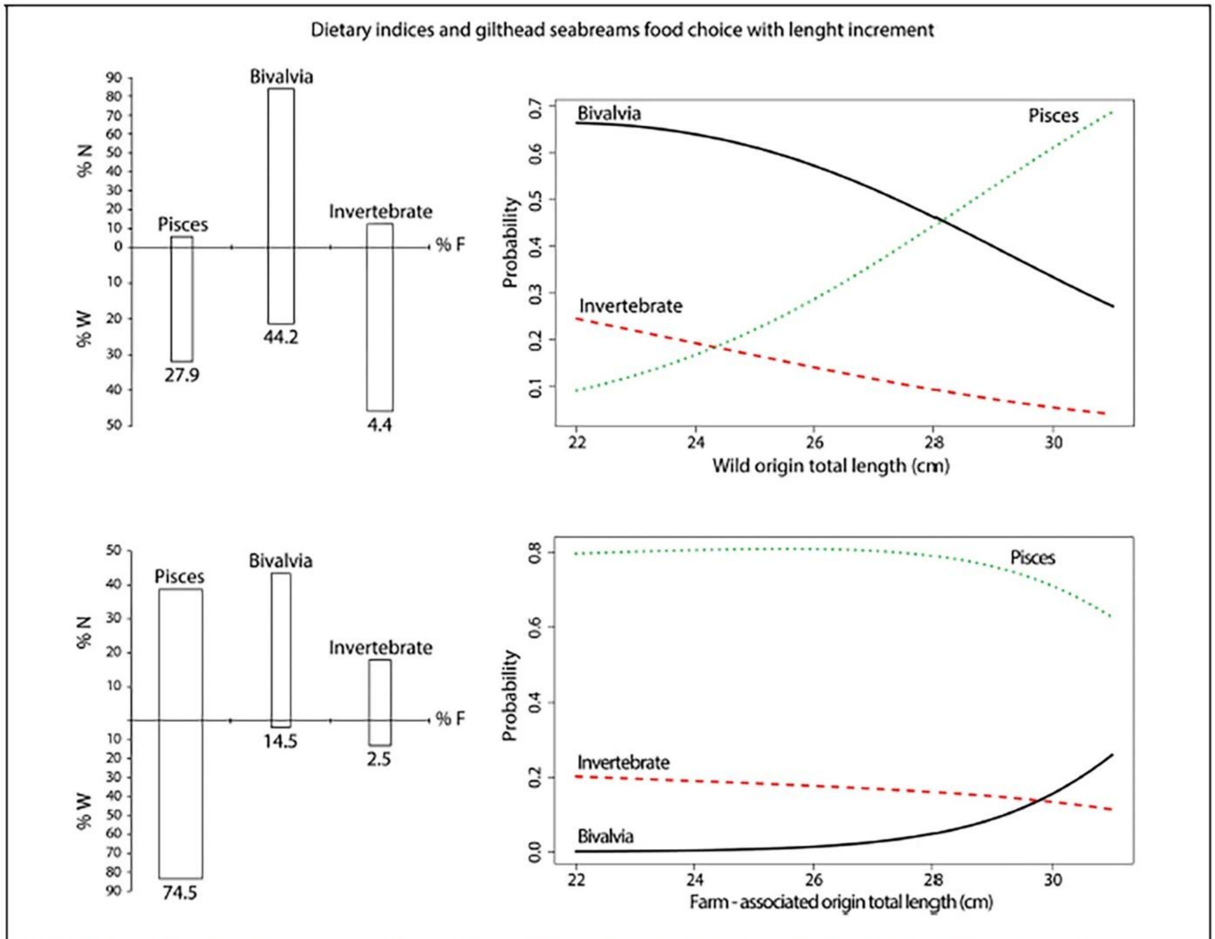

FIGURE 5 | Estimated multinomial regression model for food choice data derived from interaction between "Total length" and "Origin" predictor variables, with corresponding dietary indices of frequency of occurrence (% F), precent number (% N), and weight (% W) for the major prey groups in the diet. The predicted probabilities are plotted against total length of the gilthead seabream specimens by origin for different categories of the food outcome variable.

the species reared and feed used, it seems that farms elicit similar patterns of plastic responses in aggregated fish. It has been demonstrated that favourable ecological conditions promote the expression of plasticity in phenotypic traits (Van Buskirk and Steiner, 2009) and, in case of fish farms, the high resource availability generates greater variance in body shape traits. Consequentially, this initiated the process of morphological divergence between farm-associated fish and fish of wild origin. On the contrary, scarcer foraging conditions in natural niches promote a less plastic wild body shape by maintaining lower morphological variance and characteristics associated with the gilthead seabream durophagy eating behaviour.

Based on the quantitative model applied here that aimed to evaluate food choice trends of fish in accordance with origin and size, the farm-associated seabream showed a consistently higher probability of choosing fish as the main pray in relation to fish of wild origin, where a transition was noted from bivalves to fish prey as the preferred food type with increasing size (see **Figure 5**). The observed food choices reflected the optimal foraging behaviour concept on the one hand, and energy optimisation linked with reproduction success on the other (Townsend and Winfield, 1985). Namely, the predator maximises energy gains by evaluating prey handling time and its abundancy in the environment, i.e., the basic prey model (Gill, 2003). Due to an unlimited source of baitfish around farms (Šegvić-Bubić et al., 2011a; Stagličić et al., 2017; Fernandez-Jover et al., 2020), as a soft-textured prey rich in macro- and micronutrients that can be easily handled (Andrew et al., 2003), farm-associated seabream have favoured prey selectivity towards fish, despite the high quantities of biofouling communities on cage nets available as an alternative food source. For wild seabream, the diet is primarily based on bivalves, arthropods, and gastropods (Pita et al., 2002; Šegvić-Bubić et al., 2011a), and the modelled transition from bivalves to fish prey coincides with length sizes when gonad maturation and spawning of females occurs, explaining the shift

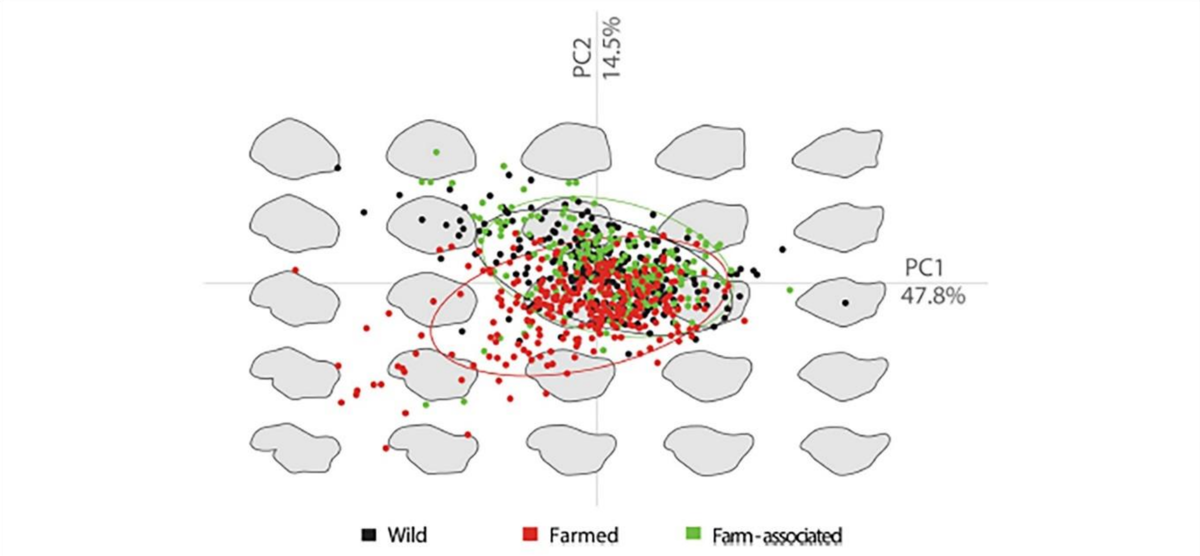

FIGURE 6 | Principal component analysis plot with 95% confidence ellipses depicting the gilthead seabream otolith morphological variation based on elliptic Fourier descriptors.

in feeding behaviour towards a diet with a higher energy content that is required for successful reproduction (Lloret et al., 2014).

Impact of Diet on Flesh Quality Composition

In conjunction with the stomach content analysis, biochemical composition of tissue provided an additional insight into the trophic status of gilthead seabream and assisted in the detection of resource polymorphisms found in this dataset. Farmed seabream had a higher lipid and lower water content compared to the other two origins (see **Supplementary Table S7**), as a result of the high fat level intake and reduced activity in cultured conditions (Alasalvar et al., 2002). The increased levels of oleic and linoleic acid and a low n-3/n-6 ratio found in farmed seabream confirms their usefulness as indicators of pellet feed consumption, due to the inclusion of vegetable oils in fish feed. Namely, fatty acids of terrestrial origin are traceable due to their low natural presence in marine organisms (Arechavala-Lopez et al., 2013b). Also, higher values of EPA, DHA and n-3 fatty acids observed in farm-associated seabream in comparison to wild fish (see **Supplementary Table S7**) reflect the rich omega-3 fatty acid content of consumed baitfish (Mourente and Tocher, 2009), even though bivalves, as the primary prey of wild populations, are a good source of high quality lipids (Tan et al., 2020). These fatty acids are essential nutrients needed for optimal reproduction of gilthead seabream (Moretti et al., 1999). A transference of somatic energy for acquiring higher reproductive potential in populations associated with tuna farms has been reported, with a deviation from the known protandry sex reversal pattern, where functional females occurred at smaller body lengths than in wild individuals (Stagličić et al., 2017; Talijančić et al., 2019). Due to the complexity of fish reproduction, it is difficult to determine whether an increase in essential fatty acid content promotes the reproductive performance of resident populations. It appears that seabream possesses the ability to selectively forage those trophic resources that are beneficial for successful reproduction, especially during the spawning period when seabream continues to feed (Fernández-Palacios et al., 2011).

TABLE 4 | Leave-one-out cross validation table of classified seabream according to fish origin, based on the otolith shape characteristics.

Origin	Wild	Farmed	Farm-associated
Wild	225 (70.98%)	23 (7.26%)	69 (21.77%)
Farmed	17 (5.8%)	256 (87.37%)	20 (6.82%)
Farm-associated	126 (54.3%)	33 (14.22%)	73 (31.47%)

External Body Colour and Otolith Appearance Features

Differences in skin pigmentation between wild and farmed origins were recognised when image-based colour analysis was performed (see colour clusters I and II in **Figure 3**). Farmed fish exhibited a higher percentage of darker silvery-grey hues and their coverage on the (i) head region, particularly around the golden band between eyes; (ii) enlarged black notch at the origin of the lateral line; (iii) upper body area, where an increased number of dark longitudinal lines was recorded on the flanks, and on the (iv) dorsal, caudal and pectoral fins. To the best of our knowledge, this is the first study to describe the main colouration patterns along the body surface using automatic and non-invasive image analysis, and the results indicate it is a usable tool for wild and farmed fish stock discrimination. The

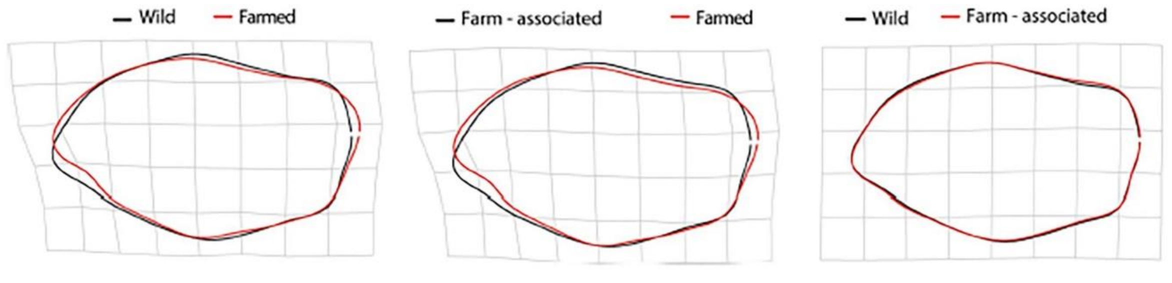

FIGURE 7 | Otolith deformation grids obtained from thin plate splines (TPS) analysis between mean shapes of wild, farm-associated and farmed origin.

results presented here are consistent with the findings where qualitative colour analyses were applied. Wild seabream were described as ones that exhibited vivid pigmentation, whereas farmed fish were reported as either with a pale greying or a darken skin description (Grigorakis et al., 2002; Rogdakis et al., 2011; Šimat et al., 2012). It seems that farming conditions, including high fish densities, daylight exposure and commercial feed deficient in natural sources of pigments, triggers an excessive production of melanin (Pavlidis et al., 2011). As a result, farmed fish show high colouration similarity and a typically dark farmed appearance, regardless of broodstock source and farming location, as observed in this study (see **Table 2**, **Figures 4**, and **Supplementary Figure 5**).

Interestingly, resident populations in the vicinity of tuna farms showed a greater colour similarity with the farmed group (46.09%) than with wild counterparts (26.28%), displaying the presence of a paler main body colour. It can be argued that due to foraging behaviour, where farm-associated individuals actively chose fish over other prey, the adequate carotenoid content needed for exhibiting iridescent and vivid colour hues is not achieved from the diet, as in case of wild fish having a carotenoid rich diet based on bivalves and other invertebrates. Inclusion of natural carotenoid sources in a low fishmeal diet can improve skin pigmentation of farmed seabream, especially with a mixture of dried marine microalgae and red swamp crayfish meal (Pulcini et al., 2020).

In combination with morphological body features, otolith shape is widely used as an effective tool for stock discrimination (Afanasyev et al., 2017), even for different origins growing under the same environmental conditions (Cardinale et al., 2004; Vignon and Moran, 2010). It has been recognised that otolith shape reflects an interplay of environmental, genetic and ontogenetic influences. In the case of gilthead seabream, this is an informative phenotypic tracer for farmed vs. wild fish recognition (Arechavala-Lopez et al., 2013b). In the present study, we observed an otolith specific shape response corresponding to fish origin when 56 Fourier descriptors were included, despite the high variability of the anterior–ventral notched part found in farmed fish that induced diversification of the rostrum and antirostrum structures (**Figure 6**, **Supplementary Figure 7**). Still, an indentation of the excisura area was found to be the main mean shape trait responsible for successful classification of individuals to their origin, when farmed (87%) and wild fish (71%) were considered, respectively. A similar farmed vs wild differentiation pattern in mean otolith shape of gilthead seabream has been reported elsewhere (Geladakis et al., 2020), suggesting that environmental conditions, including an altered diet, play the primary role in the formation of a farmed-specific otolith shape, while the genetic background was important to a lesser extent. Considering that the 70% level of successful classifications, this supports a hypothesis of separate fish stocks (Harbitz and Albert, 2015), and the applied image-based techniques and EFA methods in this study proved to be a reliable tool for wild-farmed origin delineation (see also Geladakis et al., 2020). Farm-associated individuals showed the lowest classification rate (31%) to its origin, with half of individuals classified as being of wild origin, as expected, due to limited shape variations between the origins. Only the ventral edge and postrostrum area varied, producing the rounder mean shape in FA individuals (**Figures 6**, **7**). For several marine species, Mille et al. (2016) showed variations both in global shape, such as the degree of ellipticity, and in finer details, such as otolith crenation or the width of the *excisura major*, with intrapopulation differences in the taxonomic prey categories consumed. Authors suggested that protein composition in the saccular endolymph, which varies in accordance with the type of prey digested, contributes in the synthesis of otolith matrix proteins involved in the control of crystal structure. Thus, the dietary differences of farm-associated and wild fish (ensuing from a fish vs. bivalve-based diet) could explain the shape variations found in the present study.

CONCLUSION

The multidisciplinary approach employed here revealed environmentally responsive plastic traits in populations of gilthead seabream, separating individuals of wild, farm-associated, and farmed origin when colouration, feeding behaviour and traits in body and otolith shape were considered. As farmed populations have been modified by their cultured environment and artificial selection, so have the farm-associated populations been shaped by their interactions with tuna farms, particularly with cues such as resource availability and local hydrodynamics. Still, to fully understand the different phenotypic outcomes of farm-associated gilthead seabream and whether this can be considered a distinctive morph stemming from resource polymorphism, the associated molecular mechanisms, both genetic and epigenetic, involved in the regulation of phenotypically plastic traits, should be studied. Whether these plastic traits are subject to selection and are ultimately manifested as an adaptive phenotype through quantitate genetic changes remains a challenge for future work. Concerning the applied methodologies, digital technology has proven useful in depicting the external appearance through images, for both body shape and colour analysis, respectively. Simultaneous acquisition of different traits increases the discriminating power and applicability of morphometric techniques as tools for controlling escape events within wild populations or fisheries landings. However, the degree to which phenotypic plasticity allows the farmed phenotype to (re)converge on a wild-type phenotype over time in the natural environment is still unknown, especially for the individuals escaping in early ontogenetic stages (Rogdakis et al., 2011; Arechavala-Lopez et al., 2013a). Thus, otolith shape presents a more useful phenotypic tracer due to its solid structure in comparison with environmentally plastic body features. Since escaped fish have an ecological and socioeconomic footprint on the environment and fisheries, these findings may be beneficial in allowing for more effective traceability of escapees in the wild.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because fish samples were sourced from commercial fisheries.

AUTHOR CONTRIBUTIONS

IT and TŠ-B conceived the study. IT, IŽ, LG, VK, and TŠ-B conducted the sampling. JŠ conducted stomach content analyses. JP proximated composition and fatty acid determination. IT performed the statistical data analyses. IT, TŠ-B, and IŽ wrote and revised the manuscript. TŠ-B obtained funding for the work. All authors have reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.694627/full#supplementary-material

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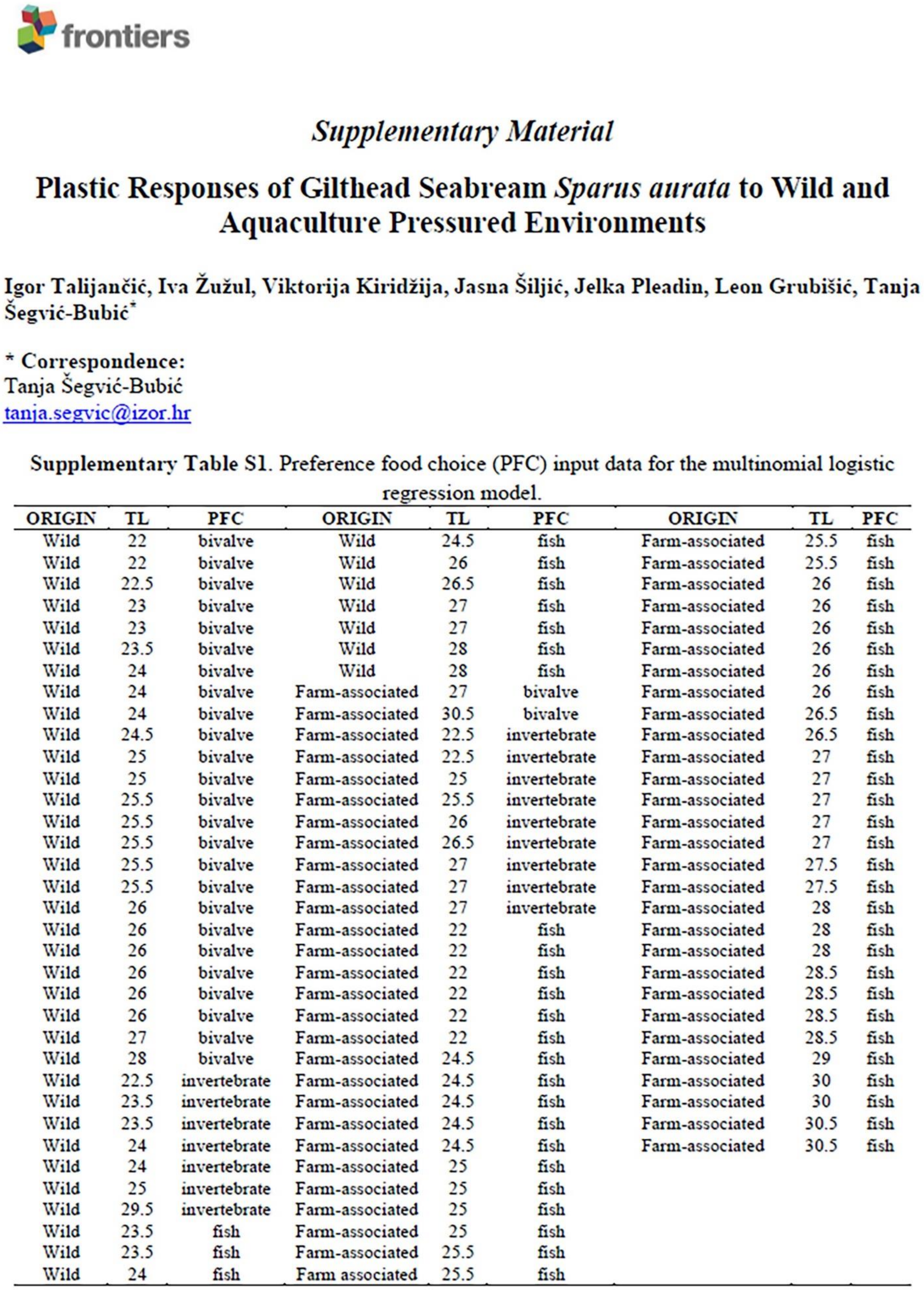
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



Supplementary Material

Plastic Responses of Gilthead Seabream *Sparus aurata* to Wild and Aquaculture Pressured Environments

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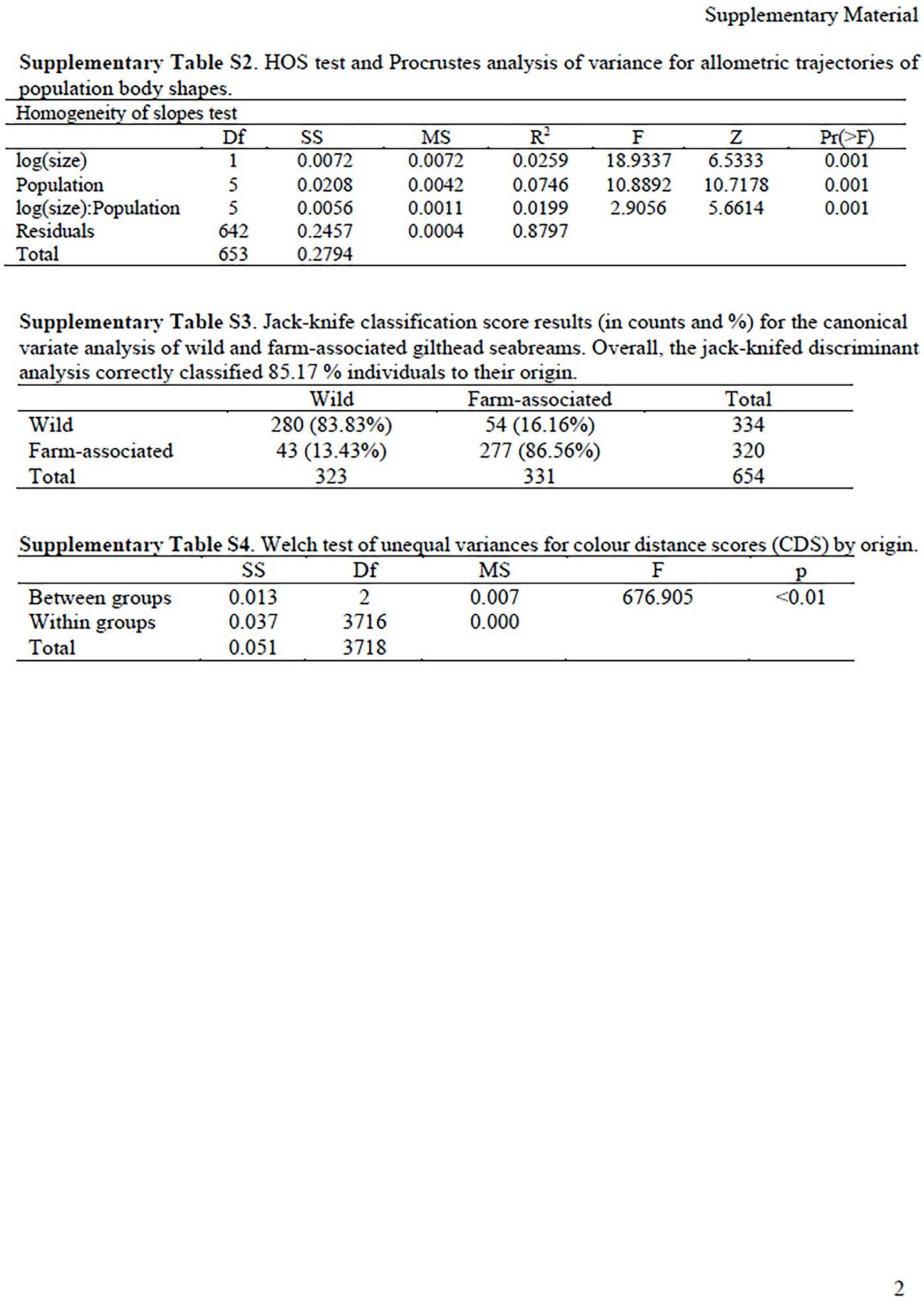
Supplementary Table S1. Preference food choice (PFC) input data for the multinomial logistic regression model.

ORIGIN	TL	PFC	ORIGIN	TL	PFC	ORIGIN	TL	PFC
Wild	22	bivalve	Wild	24.5	fish	Farm-associated	25.5	fish
Wild	22	bivalve	Wild	26	fish	Farm-associated	25.5	fish
Wild	22.5	bivalve	Wild	26.5	fish	Farm-associated	26	fish
Wild	23	bivalve	Wild	27	fish	Farm-associated	26	fish
Wild	23	bivalve	Wild	27	fish	Farm-associated	26	fish
Wild	23.5	bivalve	Wild	28	fish	Farm-associated	26	fish
Wild	24	bivalve	Wild	28	fish	Farm-associated	26	fish
Wild	24	bivalve	Farm-associated	27	bivalve	Farm-associated	26	fish
Wild	24	bivalve	Farm-associated	30.5	bivalve	Farm-associated	26.5	fish
Wild	24.5	bivalve	Farm-associated	22.5	invertebrate	Farm-associated	26.5	fish
Wild	25	bivalve	Farm-associated	22.5	invertebrate	Farm-associated	27	fish
Wild	25	bivalve	Farm-associated	25	invertebrate	Farm-associated	27	fish
Wild	25.5	bivalve	Farm-associated	25.5	invertebrate	Farm-associated	27	fish
Wild	25.5	bivalve	Farm-associated	26	invertebrate	Farm-associated	27	fish
Wild	25.5	bivalve	Farm-associated	26.5	invertebrate	Farm-associated	27	fish
Wild	25.5	bivalve	Farm-associated	27	invertebrate	Farm-associated	27.5	fish
Wild	25.5	bivalve	Farm-associated	27	invertebrate	Farm-associated	27.5	fish
Wild	26	bivalve	Farm-associated	27	invertebrate	Farm-associated	28	fish
Wild	26	bivalve	Farm-associated	22	fish	Farm-associated	28	fish
Wild	26	bivalve	Farm-associated	22	fish	Farm-associated	28	fish
Wild	26	bivalve	Farm-associated	22	fish	Farm-associated	28.5	fish
Wild	26	bivalve	Farm-associated	22	fish	Farm-associated	28.5	fish
Wild	26	bivalve	Farm-associated	22	fish	Farm-associated	28.5	fish
Wild	27	bivalve	Farm-associated	22	fish	Farm-associated	28.5	fish
Wild	28	bivalve	Farm-associated	24.5	fish	Farm-associated	29	fish
Wild	22.5	invertebrate	Farm-associated	24.5	fish	Farm-associated	30	fish
Wild	23.5	invertebrate	Farm-associated	24.5	fish	Farm-associated	30	fish
Wild	23.5	invertebrate	Farm-associated	24.5	fish	Farm-associated	30.5	fish
Wild	24	invertebrate	Farm-associated	24.5	fish	Farm-associated	30.5	fish
Wild	24	invertebrate	Farm-associated	25	fish			
Wild	25	invertebrate	Farm-associated	25	fish			
Wild	29.5	invertebrate	Farm-associated	25	fish			
Wild	23.5	fish	Farm-associated	25	fish			
Wild	23.5	fish	Farm-associated	25.5	fish			
Wild	24	fish	Farm associated	25.5	fish			



Supplementary Table S2. HOS test and Procrustes analysis of variance for allometric trajectories of population body shapes.

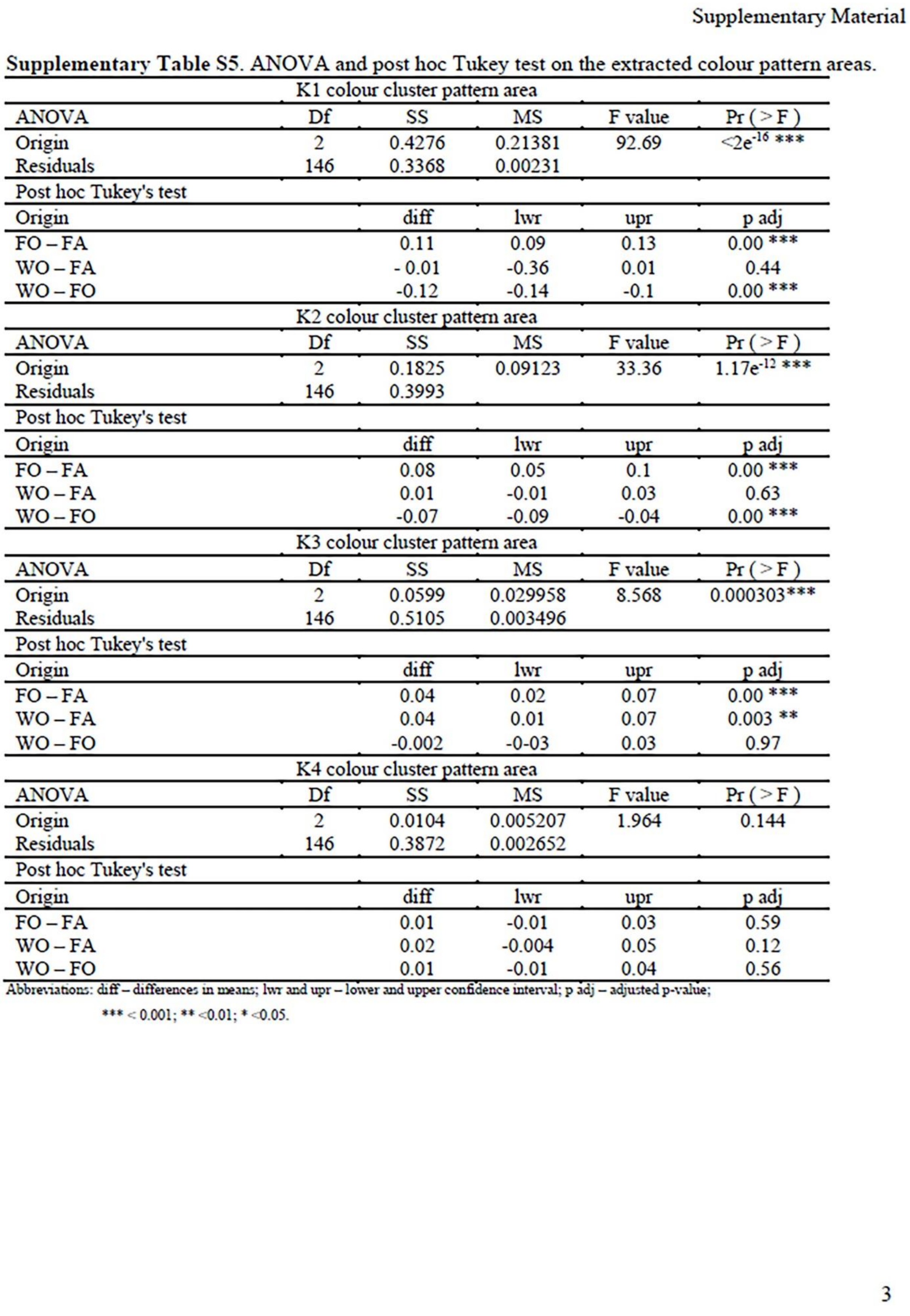
Homogeneity of slopes test							
	Df	SS	MS	R^2	F	Z	Pr(>F)
log(size)	1	0.0072	0.0072	0.0259	18.9337	6.5333	0.001
Population	5	0.0208	0.0042	0.0746	10.8892	10.7178	0.001
log(size):Population	5	0.0056	0.0011	0.0199	2.9056	5.6614	0.001
Residuals	642	0.2457	0.0004	0.8797			
Total	653	0.2794					

Supplementary Table S3. Jack-knife classification score results (in counts and %) for the canonical variate analysis of wild and farm-associated gilthead seabreams. Overall, the jack-knifed discriminant analysis correctly classified 85.17 % individuals to their origin.

	Wild	Farm-associated	Total
Wild	280 (83.83%)	54 (16.16%)	334
Farm-associated	43 (13.43%)	277 (86.56%)	320
Total	323	331	654

Supplementary Table S4. Welch test of unequal variances for colour distance scores (CDS) by origin.

	SS	Df	MS	F	p
Between groups	0.013	2	0.007	676.905	<0.01
Within groups	0.037	3716	0.000		
Total	0.051	3718			



Supplementary Table S5. ANOVA and post hoc Tukey test on the extracted colour pattern areas.

K1 colour cluster pattern area					
ANOVA	Df	SS	MS	F value	Pr (> F)
Origin	2	0.4276	0.21381	92.69	$<2e^{-16}$ ***
Residuals	146	0.3368	0.00231		
Post hoc Tukey's test					
Origin		diff	lwr	upr	p adj
FO – FA		0.11	0.09	0.13	0.00 ***
WO – FA		- 0.01	-0.36	0.01	0.44
WO – FO		-0.12	-0.14	-0.1	0.00 ***
K2 colour cluster pattern area					
ANOVA	Df	SS	MS	F value	Pr (> F)
Origin	2	0.1825	0.09123	33.36	$1.17e^{-12}$ ***
Residuals	146	0.3993			
Post hoc Tukey's test					
Origin		diff	lwr	upr	p adj
FO – FA		0.08	0.05	0.1	0.00 ***
WO – FA		0.01	-0.01	0.03	0.63
WO – FO		-0.07	-0.09	-0.04	0.00 ***
K3 colour cluster pattern area					
ANOVA	Df	SS	MS	F value	Pr (> F)
Origin	2	0.0599	0.029958	8.568	0.000303***
Residuals	146	0.5105	0.003496		
Post hoc Tukey's test					
Origin		diff	lwr	upr	p adj
FO – FA		0.04	0.02	0.07	0.00 ***
WO – FA		0.04	0.01	0.07	0.003 **
WO – FO		-0.002	-0-03	0.03	0.97
K4 colour cluster pattern area					
ANOVA	Df	SS	MS	F value	Pr (> F)
Origin	2	0.0104	0.005207	1.964	0.144
Residuals	146	0.3872	0.002652		
Post hoc Tukey's test					
Origin		diff	lwr	upr	p adj
FO – FA		0.01	-0.01	0.03	0.59
WO – FA		0.02	-0.004	0.05	0.12
WO – FO		0.01	-0.01	0.04	0.56

Abbreviations: diff – differences in means; lwr and upr – lower and upper confidence interval; p adj – adjusted p-value;

*** < 0.001; ** <0.01; * <0.05.

Supplementary Table S6. Gilthead seabream diet composition. Dietary indices used: % frequency occurrence (%F), % by number (%N), % by dry weight (%W) and % index of relative importance (%IRI) of each prey category.

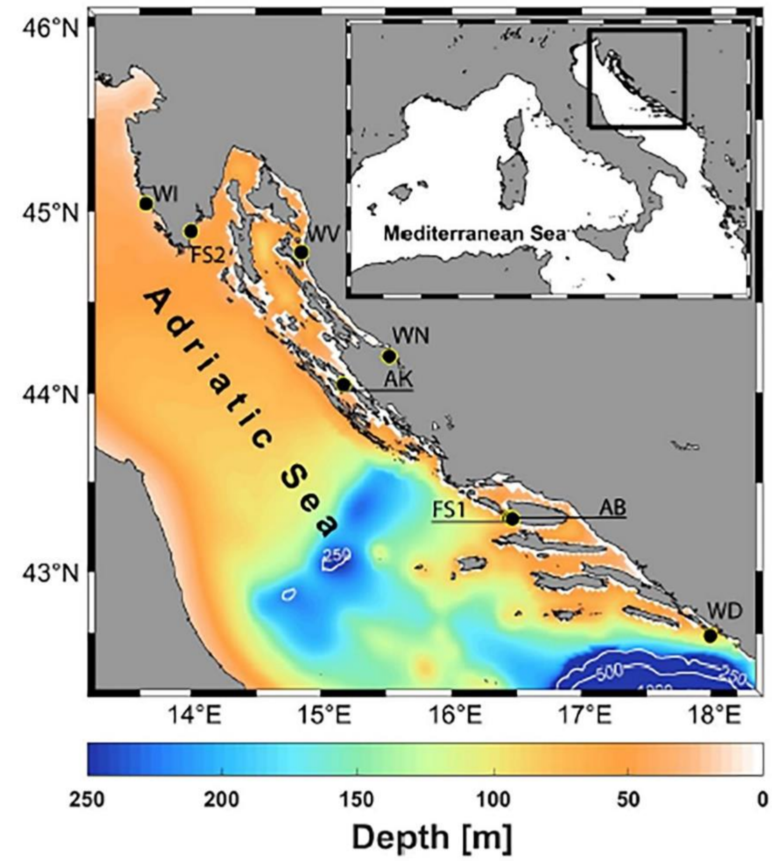
Wild origin Prey		F (%)	N (%)	W (%)	IRI (%)
Fish		0.279	0.056	0.319	16.881
Bivalves		0.442	0.828	0.222	74.973
Invertebrates		0.044	0.012	0.046	0.195
	Cephalopoda	0.000	0.000	0.000	0.000
	Tunicata	0.023	0.005	0.006	0.041
	Crustacea	0.023	0.014	0.004	0.068
	Gastropoda	0.023	0.005	0.135	0.524
	Polychaeta	0.023	0.005	0.066	0.267
	Echinodermata	0.047	0.009	0.048	0.429
	Cnidaria	0.047	0.028	0.041	0.517
	Anthozoa	0.023	0.005	0.006	0.041
	Porifera	0.023	0.005	0.0108	0.058
	Algae	0.209	0.042	0.141	0.000

Farm-associated origin Prey		F (%)	N (%)	W (%)	IRI (%)
Fish		0.745	0.387	0.836	91.922
Bivalves		0.145	0.434	0.034	6.860
Invertebrates		0.025	0.018	0.013	0.079
	Cephalopoda	0.018	0.009	0.007	0.029
	Tunicata	0.018	0.009	0.013	0.041
	Crustacea	0.018	0.009	0.002	0.021
	Gastropoda	0.018	0.009	0.016	0.047
	Polychaeta	0.018	0.009	0.007	0.029
	Echinodermata	0.073	0.038	0.030	0.497
	Cnidaria	0.018	0.028	0.004	0.059
	Anthozoa	0.018	0.009	0.032	0.076
	Porifera	0.000	0.000	0.000	0.000
	Algae	0.055	0.057	0.019	0.000

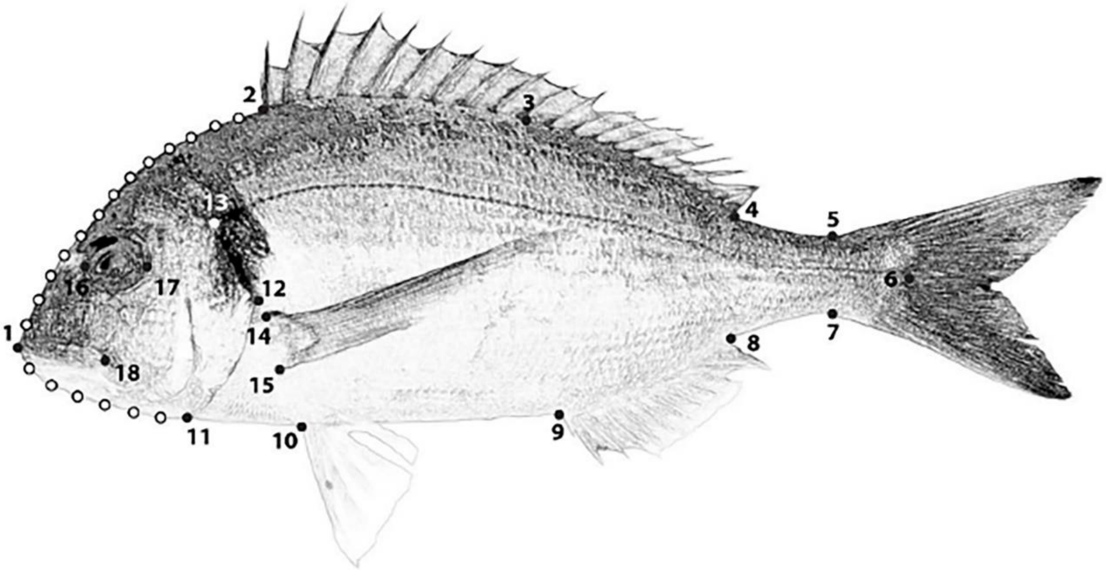
Supplementary Table S7. The proximate, mineral and fatty acid (expressed as % of total fatty acids) compositions of the sampled gilthead seabream per origin (n = 4).

Proximate and mineral composition	WO – Dubrovnik (WD)	FA – Brač tuna farm (AB)	FO – Farm site 1 (FS1)	SDM
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water (g/100g)	78.47 ± 0.3	78.58 ± 0.2	70.51 ± 0.5	76.14 ± 3.5
Protein (g/100g)	18.7 ± 0.1	18.38 ± 0.2	18.1 ± 0.1	18.42 ± 0.4
Fat (g/100g)	1.65 ± 0.2	1.72 ± 0.1	10.2 ± 0.4	4.24 ± 3.9
Ash (g/100g)	1.26 ± 0.0	1.3 ± 0.0	1.23 ± 0.0	1.26 ± 0.0
Na (mg/kg)	311 ± 2.3	300 ± 5.3	311 ± 4.3	304.33 ± 6.2
Ca (mg/kg)	278 ± 4.7	257 ± 9.4	272 ± 8.2	266.33 ± 12.7
P (mg/kg)	3464 ± 20.6	3509 ±15.4	3429 ± 21.1	3469 ± 52.2
Fatty acid composition				
C14:0 (MA)	3.6 ± 0.1	5.3 ± 0.4	2.4 ± 0.0	3.77 ± 1.2
C15:0	0.75 ± 0.05	0.9 ± 0.0	0.25 ± 0.05	0.63 ± 0.3
C16:1 (PC)	27.2 ± 0.2	27.8 ± 0.6	14.3 ± 0.2	23.1 ± 6.2
C16:1n7t	0.9 ± 0.1	0.75 ± 0.05	0.5 ± 0.0	0.72 ± 0.2
C16:1n7c (PA)	6.35 ± 0.15	9.6 ± 0.9	3.35 ± 0.1	6.43 ± 2.6
C17:0	1 ± 0.0	1.1 ± 0.0	0.2 ± 0.0	0.77 ± 0.4
C17:1	0.5 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
C18:0 (SA)	12.75 ± 0.85	10.5 ± 1	3.5 ± 0.0	8.92 ± 4.0
C18:1n9c (OA)	37.05 ± 0.65	30.85 ± 1.25	45.55 ± 0.1	37.82 ± 6.1
C18:1n7 (VA)	3.55 ± 0.1	4.1 ± 0.2	3.15 ± 0.1	3.6 ± 0.4
C18:2n6c (LA)	1.55 ± 0.8	0.7 ± 0.1	14.95 ± 0.2	5.73 ± 6.5
C18:3n3	0.1 ± 0.0	0.15 ± 0.0	2.8 ± 0.1	1.03 ± 1.3
C18:4n3	0.5 ± 0.0	0.15 ± 0.0	0.2 ± 0.0	0.28 ± 0.0
C20:0	0.55 ± 0.1	0.5 ± 0.0	0.35 ± 0.1	0.47 ± 0.1
C20:1n9	1.7 ± 0.1	1.95 ± 0.2	2.3 ± 0.1	1.98 ± 0.3
C20:2n6	0.15 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.25 ± 0.0
C20:4n6	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.23 ± 0.1
C20:5n3 (EPA)	0.1 ± 0.0	0.6 ± 0.2	0.6 ± 0.0	0.4 ± 0.3
C22:0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.23 ± 0.0
C22:1n11	0.1 ± 0.0	0.25 ± 0.0	1.05 ± 0.1	0.46 ± 0.0
C22:1n9	0.65 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.72 ± 0.1
C22:5n3	0.75 ± 0.1	0.95 ± 0.3	0.3 ± 0.0	0.67 ± 0.3
C22:6n3 (DHA)	0.1 ± 0.0	1.4 ± 0.6	0.95 ± 0.1	0.82 ± 0.6
C24:1n9	0.25 ± 0.0	0.7 ± 0.1	0.65 ± 0.1	0.58 ± 0.1
SFA	3.16 ± 0.1	3.38 ± 0.4	1.15 ± 0.0	2.56 ± 1.0
MUFA	50.55 ± 0.2	48.65 ± 0.4	56.3 ± 0.2	51.83 ± 3.3
PUFA	0.42 ± 0.1	0.7 ± 0.1	1.63 ± 0.0	0.91 ± 0.5
∑ n-3	0.91 ± 0.1	3.15 ± 0.8	5.35 ± 0.2	3.14 ± 1.8
∑ n-6	1.9 ± 0.9	0.95 ± 0.4	15.85 ± 0.2	6.23 ± 6.81
∑ n-3 / ∑ n-6	0.47	3.32	0.34	0.50
EPA / DHA	0.1	0.45	0.63	0.49

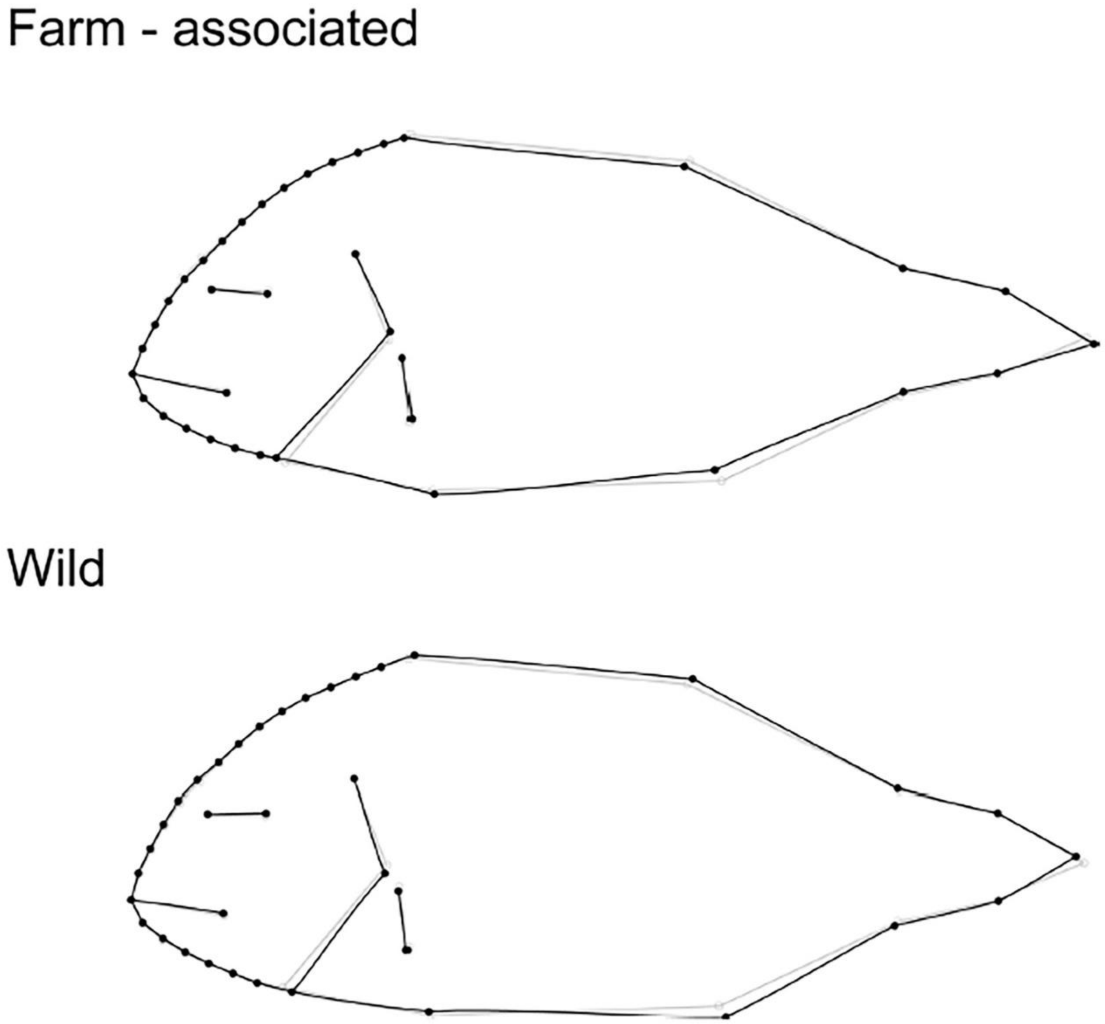
Abbreviations: WO - wild origin; FA – farm-associated origin; FO – farmed origin; MA – myristic acid; PC – palmitic acid; SA – stearic acid; OA – oleic acid; VA – vaccenic acid; LA – linoleic acid; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; SFA – saturated fatty acid; MUFA – monosaturated fatty acid; PUFA – polyunsaturated fatty acid; SDM – Standard deviation and mean of the overall sample.



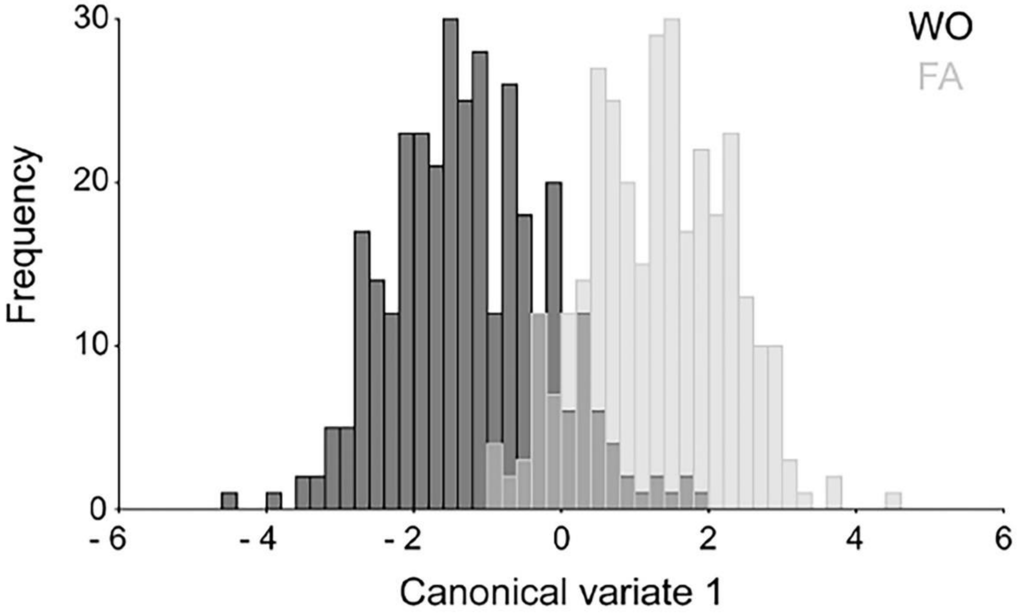
Supplementary Figure S1. Sampling locations of farmed (FS1 – Farm site 1; FS2 – Farm site 2), farm-associated (AB – Brač tuna farm; AK – Kali tuna farm) and wild *Sparus aurata* populations (WD – Dubrovnik; WI – Istria; WN – Novigrad Sea; WV – Velebit Channel) along the eastern Adriatic coast.



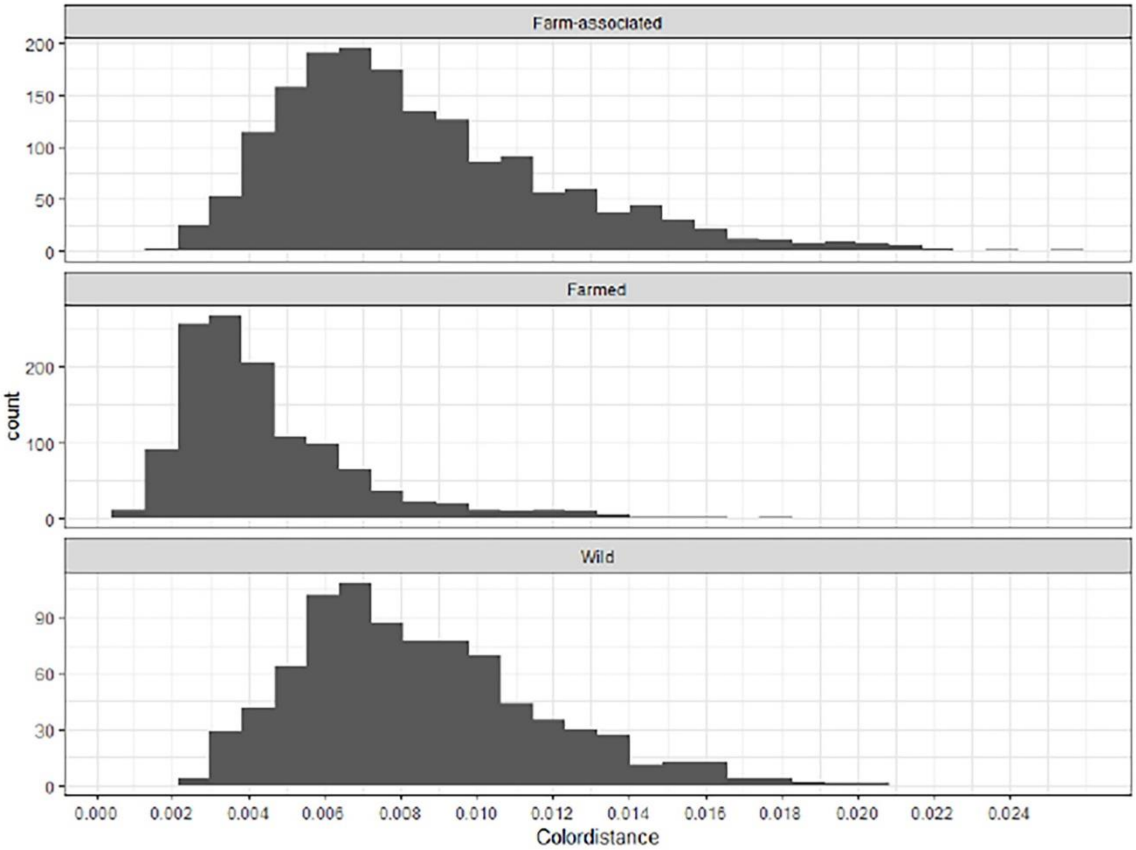
Supplementary Figure S2. Graphical representation of 18 landmarks (labelled full black or white dots) were used to visualize the body shape of the sampled *Sparus aurata*: 1) anterior tip of snout at the upper jaw; 2) anterior insertion of the dorsal fin; 3) last spiny ray of the dorsal fin; 4) posterior insertion of the dorsal fin; 5) dorsal point at the least depth of the caudal peduncle; 6) posterior body extremity; 7) ventral point at the least depth of the caudal peduncle; 8) posterior insertion of the anal fin; 9) anterior insertion of the anal fin; 10) insertion of the pelvic fin; 11) ventral tip of the insertion of the operculum on the lateral profile; 12) point of maximum extension of the operculum on the lateral profile; 13) anterior extremity of the lateral line on the head profile; 14) dorsal insertion of the pectoral fin; 15) ventral insertion of the pectoral fin; 16) the most anterior point in the eye; 17) the most posterior point in the eye; 18) posterior end of snout at the upper jaw. Curves on the head profile were sampled with 19 semilandmarks, represented as empty white circles (unlabelled).



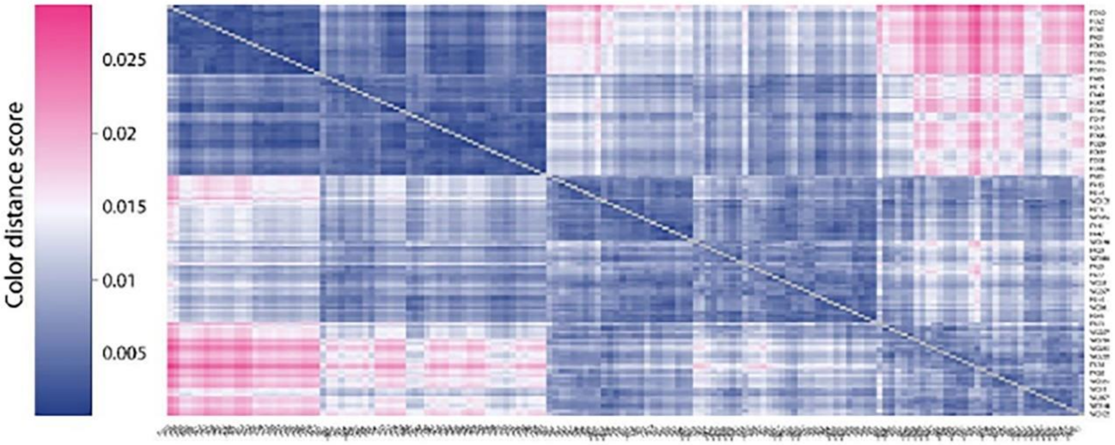
Supplementary Figure S3. Wireframe drawings of mean shapes for farm-associated and wild gilthead seabream. The grey wireframe represents the transformation from the overall mean shape, whereas the black wireframe represents the mean shape of each origin.



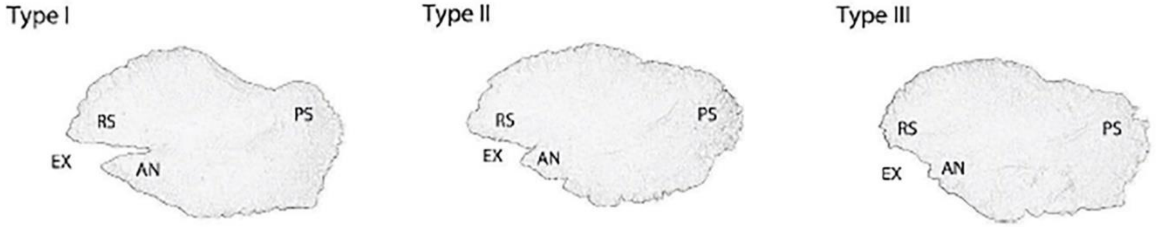
Supplementary Figure S4. Canonical variate graph of sampled origins based on Procrustes distances. Farm- associated (FA) specimens shown in grey and wild (WO) specimens in black.



Supplementary Figure S5. Distribution of colour distance scores (CDS) by fish origin.



Supplementary Figure S6. The EMD distance colour matrix of sampled images, where blue cells indicate higher similarity (lower distance), while magenta ones indicate higher dissimilarity (higher distance).



Supplementary Figure S7. Sampled farmed gilthead seabream otolith variability. The main distinguishing feature of the FO structure is the excisura (EX) or portion of the anterior margin of the otolith where the ostium opens. The anterior region varies from double-peaked with a pointed rostrum (RS) and defined antirostrum (AN; Type I and II) to a peaked region with only a shallow notch, as a small indent in the otolith margin (Type III). In the posterior region of the otolith structure, the postrostrum (PS) varies in shape from oblique (Type I and II) to nearly flattened (Type III).

RASPRAVA

Populacije riba imaju izrazitu globalnu vrijednost te pružaju i oblikuju usluge ekosustava ljudima širom svijeta (Cooke i sur., 2016). Međutim, vodeni ekosustavi izloženi su značajnim okolišnim i antropogenim promjenama, a ribe imaju ključan položaj kao pokazatelj zdravlja okoliša. Pritisci u vidu prekomjernog iskorištavanja resursa (ribarstvo, akvakultura, energija), onečišćenje zbog povećanja ljudske populacije te klimatske promjene izravno i neizravno utječu na život u moru (Ceballos i sur., 2017). Izmjena i fragmentacija staništa pod djelovanjem ribolova, akvakulture ili proizvodnje energije (platforme za bušenje nafte i plina, vjetroturbine) uzrokuju široki raspon bioloških posljedica za riblje vrste i populacije, od letalnih do fizioloških, uključujući učinke na ponašanje različitih dobnih skupina (Kuparinen i Festa-Bianchet, 2017; Naylor i sur., 2000). Svaka vrsta ima svojstven odgovor na promjene u okolišu, uvjetovan evolucijskim i demografskim procesima kao i sposobnostima prilagodbe.

U zadnja dva desetljeća s porastom akvakulture i temperature mora (Barange i sur., 2018), komarča (*Sparus aurata*, Linnaeus 1758) je postala sve brojnija vrsta u Jadranu i ostatku Sredozemlja (Glamuzina i sur., 2014). Proširila je geografsku rasprostranjenost na sjevernu hemisferu gdje je prije sporadično bilježena (Avignon i sur., 2017). U osvajanju novih staništa, vrsta učestalije obitava na uzgajalištima školjkaša duž Sredozemlja gdje se aktivno hrani školjkašima, ugrožavajući ekonomsku stabilnost sektora (Šegvić-Bubić i sur., 2011c; Richard i sur., 2020). Uz lubina, komarča je glavna vrsta u akvakulturi EU, s godišnjom stopom rasta proizvodnje od 5%. Posljedično, u divljim je populacijama komarči zabilježena prisutnost uzgojnih genotipova s prosječnim udjelom od 15 % te se očekuje da će s daljnjim razvojem akvakulture zastupljenost zbjegova biti sve veća (Šegvić-Bubić i sur., 2011a; 2017; Žužul i sur., 2019). Isti su autori uočili okupljanje komarče oko uzgajališta tuna u istočnom Jadranu te se genetska struktura tih populacija razlikovala od onih uzorkovanih u područjima bez uzgojnih sustava upućujući na prostornu izoliranost usred smanjenog protoka gena (Žužul i sur., 2019).

S obzirom na uočeni obrazac okupljanja ribljih vrsta oko uzgajališta te sve veći broj zbjegova iz akvakulture u morski okoliš, u predmetnoj doktorskoj disertaciji istraženi su (1) utjecaj tunogojilišta na fenotipska svojstva populacija komarči koje obitavaju u okolici kaveznih infrastruktura te (2) bio-ekološka obilježja populacija komarči u istočnom dijelu Jadranskoga mora, kako bi se razvio brz i precizan alat raspoznavanja divljih i uzgojnih jedinki koristeći se metodama geometrijske morfometrije. Svi su relevantni odgovori na postavljene hipoteze potkrijepljeni znanstvenim člancima koji čine sastavni dio ove doktorske disertacije.

Utjecaj uzgajališta tuna na fenotipska obilježja lokalnih populacija komarči. Istraživanjem je utvrđeno da uzgajališta tuna u istočnom Jadranu privlače prirodne ihtiopopulacije tijekom cijele godine što je dokazano značajno većom uočenom brojnosti i raznolikosti vrsta u odnosu na kontrolna područja. Stereoskopska je kamera po prvi put korištena za procjenu brojnosti i sastava ihtiopopulacija oko kaveza tuna, što je u odnosu na prethodno istraživanje Šegvić-Bubić i sur. (2011b), omogućilo vizualno promatranje cijelog vodenog stupca po dubinama. Dodatno, na svakom uzgajalištu promatrane su tri lokacije (lokacija neposredno uz kavez, lokacija 500 m od uzgajališta te lokacija 1000 m od uzgajališta koja je ujedno predstavljala kontrolnu lokaciju) kako bi se kvalitetnije sagledala prostorna varijabilnost ihtiopopulacija. Tijekom jednogodišnjeg praćenja ihtiopopulacija na dvama uzgajalištima tuna u srednjem Jadranu zamijećeno je kako se okupljene ihtiopopulacije odlikuju relativno malim brojem vrsta (24), prvenstveno iz porodica Sparidae (9 vrsta), Carrangidae (2 vrste) i Serranidae (2 vrste), s bukvom i komarčom kao najučestalijim zapaženim vrstama. Najveća brojnost i bogatstvo vrsta uočeno je ispod uzgajališta pri morskom dnu (Članak I – Tablica 1; Slika 2), dok daljnji prostorni utjecaj privlačenja ne prati gradijent udaljenosti već pokazuje opadanje samo nekoliko stotina metara od uzgajališta.

Značajna varijabilnost brojnosti i bogatstva vrsta uočena je u odnosu na promatranu dubinu i udaljenost od kaveznih infrastruktura, dok je varijabilnost između godišnjih doba (topla i hladna sezona) bila manje izražena. Veće bogatstvo vrsta u pridnenim slojevima svakako se može povezati s topografskom raščlanjenošću staništa gdje strukturalno složenija morska dna podržavaju raznovrsnije i brojnije ihtiopopulacije u odnosu na homogena pelagična staništa (Garcia-Charton i Perez- Ruzafa, 1998; Kaiser i sur., 1999; Guidetti, 2000; Anderson i Millar, 2004; Garcia-Charton i sur., 2004). U odnosu na kaveznu infrastrukturu (mreže, konopi, plutače), morsko dno dominira u površini za prihvat vegetacijskog pokrova, koji osim što sam po sebi može biti prehrambeni resurs (Bell i Hermelin-Vivien, 1983), također predstavlja i stanište za cijeli niz beskralježnjaka kojima se pridnene riblje vrste hrane (Sala, 1997).

Pored učinka kavezne infrastrukture u privlačenju ihtiopopulacije, tzv. FAD efekt, hrana namijenjena tunama u uzgoju koja dospijeva u okoliš, u vidu nepojedene hrane ili ostataka (ljuske, komadići tkiva i sl.) nastalih tijekom hranjenja tuna, zasigurno pridonosi većoj brojnosti i raznolikosti vrsta ispod kaveza u odnosu na druga kontrolna područja morskog dna (Članak I –

Tablica 1; Slika 2). Ipak, sastav i brojnost vrsta uočenih u okolici uzgajališta tuna razlikovala se od onih uočenih na pučinskim uzgajalištima bijele ribe u Sredozemlju. Učinak unosa nepojedene hrane, kao i vrsta hrane koja se unosi u okoliš vjerojatno su pokretači promjena sastava i brojnosti vrsta (Dempster i sur., 2005; Šegvić-Bubić i sur., 2011b; Bacher i sur., 2012; Ballester-Moltó i sur., 2017). Mala plava riba namijenjena ishrani tuna znatno se razlikuje od peletirane hrane korištene u uzgoju bijele ribe, i to u nutritivnom sastavu, veličini, brzini topljivosti i taloženju (Aguado-Gimenez i Garcia-Garcia, 2005; Vassallo i sur., 2006; Fernandes i sur., 2007). U odnosu na predmetno istraživanje ukupna brojnost, biomasa te broj zapaženih vrsta izraženiji su na uzgajalištima bijele ribe (Dempster i sur., 2002, 2009; Boyra i sur., 2004; Bacher i sur., 2012; Goodbrand i sur., 2013; Özgül i Angel, 2013), gdje peletirana hrana koja često sadrži dodatke poput pojačivača okusa sa svojim finijim česticama ima privlačniji učinak na prirodne ihtiopopulacije u usporedbi s malom plavom ribom na uzgajalištima tuna (Šegvić-Bubić i sur., 2011b).

Veća zastupljenost vrsta iz porodice Sparidae zapažena je u okolici uzgajališta bijele ribe (Dempster i sur., 2002, 2004, 2005; Valle i sur., 2007; Fernandez-Jover i sur., 2008; Arechavala-Lopez i sur., 2011; Ballester-Moltó i sur., 2015), ali i tuna (Šegvić-Bubić i sur., 2011b; Bacher i sur., 2012). Sličan obrazac prepoznat je i u ovom istraživanju tijekom cijele godine, što potvrđuje ulogu uzgajališta tuna kao atraktivnog stanišna za lokalne populacije vrsta iz porodice Sparidae.

Bukva je dominantno najzastupljenija vrsta na uzgajalištima tuna srednjeg Jadrana, te se aktivno hrani ostacima male plave ribe u okolici kaveza (Šegvić-Bubić i sur., 2011b; Fernandez-Jover i sur., 2020). Dok su Šegvić-Bubić i sur. (2011b) uočili veću brojnost bukve u toplijem dijelu godine kada se ujedno provodi i intenzivna hranidba uzgajanih tuna, u predmetnom istraživanju koje je obuhvaćalo ista uzgajališta tuna uočen je obrazac trajnog prisustva plovi bukvi tijekom godine, bez izražene sezonske varijabilnosti. Uočene razlike vjerojatno su uvjetovane razlikama u metodološkom pristupu kvantifikacije ihtiopopulacija gdje su Šegvić-Bubić i sur. (2011b) istraživali strukturu vrsta unutar prvih 15m vodenog stupca te su za procjenu brojnosti koristili par ronioca. Naime, primjena stereoskopske kamere u predmetnom istraživanju omogućila je pristup većim dubinama (50-60m) koje su rizične za ronioce s autonomnom ronilačkom opremom. Kamerom je zabilježeno 1,5 puta učestalija pojavnost bukve pri dnu ispod uzgajališta tuna nego na morskoj površini, što objašnjava gore navedene razlike u sezonskoj raspodjeli bukve.

Među ostalim vrstama iz porodice Sparidae, u predmetnom istraživanju uočena je trajna prostorna i vremenska zastupljenost komarče oko uzgajališta tuna (Članal I – Tablica 1; Slika 3), upućujući na važnu ulogu jadranskih uzgajališta tuna kao pogodnih staništa za divlje komarče. Analizom oblika tijela komarči koje su ciljano uzorkovane oko uzgajališta tuna tijekom dvogodišnjeg razdoblja uočena je specifična tjelesna morfologija, različita od one uočene kod divljih populacija, te im je pripisan novi kavezno-povezani morfotip.

Pojavnost morfotipova uslijed promjena u ekologiji ishrane i načinu korištenja staništa, koje odlikuje specifična morfologija, obojenosti ili ponašanje, obilježje je fenotipske plastičnosti uvjetovano trofičkim polimorfizmom (Skúlason i Smith, 1995; Skúlason i sur., 2019). U odnosu na divlju komarču, kavezno-povezane jedinke u prosjeku imaju vretenastije tijelo na kojemu se nalazi manja i blago tuplja glava s povišenijim ustima (Članak III – Slika S3). Konfiguracija od 18 homolognih anatomskih točaka ostvarila je klasifikacijsku točnost od 77 % jedinki shodno divljem ili kavezno-povezanom morfotipu (Članak II – Slika S1), unatoč tome što tjelesne razlike nisu značajno izražene kao što je to slučaj između divljih i uzgojnih jedinki (Članak II – Tablica 2, Slika 2). Međutim, bolji rezultat (88 %) postignut je detaljnijim uzorkovanjem profila glave kroz uključivanje dodatnih 19 točaka krivine (Članak III - Slika S1, S4).

Trofički resursi imali su značajan utjecaj na cjelokupnu morfologiju komarče jer se tip, dostupnost i količine hrane razlikuju u prirodnim i kavezno-povezanim okolišima uzgajališta tuna. Komarča je priobalna, pridnena vrsta koja je u Jadranu rasprostranjena uz obalu, gdje obitava iznad pjeskovitih ili pjeskovito-ljušturnih dna mirnih uvala, zaljeva, ušća rijeka i naselja livada morskih cvjetnica. Odrasle jedinke obično obitavaju u obalnim područjima do 30 m dubine ili pliće, gdje su obično u potrazi za tvrdoljušturnim beskralježnjacima kao glavnim plijenom (Dulčić i Kovačić, 2020). U usporedbi s prirodnim staništima, uzgajališta tuna pružaju dodatnu strukturalnu složenost te izvor hrane u područjima gdje su veća strujanja mora zbog kriterija odabira lokacije uzgoja (Šegvić-Bubić i sur., 2011b). U ovom istraživanju veća brojnost i učestalost pojavljivanja ribe kao glavnog plijena zapažena je kod kavezno-povezanih jedinki (% IRI = 92), dok su školjkaši bili glavni plijen divljih komarči (% IRI = 75) (Članak III – Slika 5). Zastupljenost ribe kao glavnog plijena u želucima kavezno-povezanih komarči pokazatelj je oportunističkog načina hranidbe gdje vrsta prilagođava svoju ishranu ovisno o dostupnim trofičkim resursima u staništu (Pita i sur., 2002; Arechavala-Lopez i sur., 2012b). Slični obrasci ishrane zabilježeni su i kod bukve, ali ne i kod iglice (*Belone belone,* Linnaeus 1760) u blizini jadranskih uzgajališta tuna (Fernandez-Jover i sur., 2020), što potvrđuje značenje male plave ribe kao glavnog izvora hrane za okupljanje vrste iz porodice Sparidae.

Pored uočenih prosječnih razlika u tjelesnim obilježjima, relativnom analizom glavnih komponenti (relPCA) zabilježena je i dvostruko veća varijabilnost u obliku glave, orijentaciji usta te položaju očiju i parnih peraja unutar kavezno-povezanih populacija naspram divljima (Članak III – Slike 1, 2). Tijelo kavezno-povezane komarče vretenastije je u odnosu na divlju, sa širim anteriornim dijelom tijela koji se sužava prema repnoj regiji (Članak III – Slika S3). Spomenuto tjelesno obilježje omogućava učinkovitiju potragu za plijenom te pokazuje prilagodbu koja u hidrodinamičnom okolišu ima prednost nad drugim tjelesnim oblicima (Bracciali i sur., 2016). Veća varijabilnost u poziciji parnih peraja ukazuje na prilagodbu lokomotornog sustava prema odabiru ribe kao glavnog plijena. Prsne peraje kontroliraju manevarske pokrete skretanja, vertikalnog podizanja tijela kroz vodeni stupac, ubrzavanja (npr. hvatanje plijena ili bježanje od predatora), kočenja, plivanja unatrag te lebdenja (Drucker i sur., 2006). Dok divlje komarče uglavnom brste plijen u prirodnim staništima (Kara i Quignard, 2019), kavezno-povezane jedinke češće upotrebljavaju širi raspon pokreta parnih peraja kako bi se manevri hvatanja inertnog plijena, u ovom slučaju ostaci nepojedene ribe, izveli u bilo kojoj ravnini vodenog stupca (Webb, 1984). Shodno tome, varijabilnost položaja usta, od subterminalnog prema terminalnom, kao i u obliku glave (Članak III – Slika 2A) predstavlja odgovor plastičnosti na plijen pelagičnog tipa koji se uglavnom usisava od dolje prema gore, a ne drobi primjenom sile kao kod prehrane pridnenim tvrdoljušturnim beskralježnjacima, poput školjkaša ili rakova.

U odnosu na divlje populacije, manja glava s tupljom gubicom fenotipsko je svojstvo koje je uočeno i kod drugih vrsta riba iz porodice ljuskavki koje obitavaju u okolici uzgajališta bijele ribe (Abaad i sur., 2016). Izgleda da energetski bogata staništa, poput uzgajališta riba, potiču slične obrasce plastičnih odgovara kod kavezno-povezanih ihtiopopulacija, bez obzira na vrstu hrane koja se unosi u okoliš (pelet *vs*. sitna plava riba). S druge strane, plastičnost organizama manje je izražena u prirodnim staništima zbog veće varijabilnosti u odabiru plijena, uslijed kompeticije za nužne resurse i rizika od predacije (Van Buskirk i Steiner, 2009).

Uz tjelesna obilježja, zabilježen je utjecaj kavezno povezanog okoliša i na oblik otolita. Naime, oblik otolita između divljih i kavezno-povezanih jedinki razlikovao se u anteriornom i posteriorno-dorzalnom području (Članak III – Slika 7). Međutim, zbog niske razine varijabilnosti oblika otolita između promatranih grupa, klasifikacijski test linearne diskriminantne analize

(LDA) ispravno je pridružio samo 31,47 % jedinki u grupu komarči iz akvatorija uzgajališta tuna (Članak III – Tablica 4, Slika 6). Mille i sur. (2016) kod nekolicine morskih vrsta uočili su učinak različitog plijena na intrapopulacijsku varijabilnost oblika otolita, jer sastav bjelančevina ima važnu ulogu u sintezi strukture, a time povezanog oblika otolita. Za pretpostaviti je da razlike u ishrani komarči oko uzgajališta tuna naspram komarči iz prirodnih staništa, i s time povezane razlike u sastavu bjelančevina glavnih vrsta pljenova, prvenstveno ribe naspram školjkaša, predstavljaju izvor varijabilnosti oblika otolita između spomenutih grupa.

U predmetnom je istraživanju također ispitivana vjerojatnost odabira plijena u odnosu na ukupnu dužinu (TL) kavezno-povezanih i divljih komarči multinominalnom logističkom regresijom. Naime, kavezni okoliš obiluje različitim trofičkim izvorima atraktivnim za prirodne ihtiopopulacije, uključujući ostatke nepojedene male plave ribe te mnogobrojne obraštajne zajednice na kaveznim infrastrukturama gdje često prevladavaju školjkaši poput dagnje, kamenice i male kapice (*Chlamys varia*, Linnaeus 1758). Iako su različiti izvori hrane jednako dostupni u akvatoriju uzgoja, njihov je nutritivni sastav i energetski doprinos različit. Rezultati multinominalne regresije pokazali su da je odabir ribe kao glavnog plijena bio stalan kod kavezno-povezanih komarči, bez obzira na tjelesnu dužinu ribe, dok je kod divljih jedinki uočena promjena vjerojatnosti odabira glavnoga plijena porastom dužine ribe, i to od školjkaša prema ribi (Članak III – Tablica 3; Slika 5).

Pored uočenih razlika u odabiru plijena komarči, multinominalnom logističkom regresijom zapažena je i promjena spola od mužjaka prema ženki pri manjim ukupnim tjelesnim dužinama kod populacija oko uzgajališta tuna naspram onih iz prirodnih staništa (Članak II – Tablica 4, Slika 6). Obrazac promjene spola komarči duž istočnog Jadrana, gdje većina dvogodišnjih i trogodišnjih jedinki postaju funkcionalne ženke pri dužinama od 30 cm (Dulčić i Kovačić, 2020), potvrđen je i ovim istraživanjem. Odabir energetski bogatijeg plijena tijekom rasta, od beskralježnjaka k ribi, poklapa se s dužinama (~30 cm) pri kojima započinje promjena spola u prirodnim uvjetima (Članak II – Slika 6; Članak III – Slika 5). S druge strane, stalna dostupnost male plave ribe u okolici uzgajališta tuna vjerojatno je potencirala otklon od prirodnog obrasca u ishrani kod kavezno-povezane komarče. U nutritivnom pogledu sitna plava riba, kao glavni plijen komarče oko uzgajališta, osigurava važan izvor hranjivih tvari, posebice bjelančevina i lipida, uključujući skupinu esencijalnih masnih kiselina. Izgleda da komarča kao vrsta ima sposobnost selektivnog odabira onih trofičkih resursa koji pospješuju reprodukciju, pogotovo tijekom razdoblja mrijesta kada se i dalje nastavlja hraniti (Fernández-Palacios i sur., 2011).

Sastav lipida u tkivima ihtiopopulacija koje obitavaju u okolici uzgajališta bijele ribe često odražava sastav lipida koji se nalazi u hrani za ribe u uzgoju, koja sadrži visoki udio masnih kiselina biljnog podrijetla, a relativno niski udio nezasićenih omega-3 masnih kiselina (Fernandez-Jover i sur., 2007, 2011; Arechavala-Lopez i sur., 2011; Izquierdo-Gómez i sur., 2014; Uglem i sur., 2014). U ovom istraživanju, profil masnih kiselina mišićnog tkiva kavezno-povezanih komarči sadržavao je povećane vrijednosti eikosapentaenske (EPA), dokosaheksaenske (DHA) i n-3 masnih kiselina u odnosu na divlje populacije (Članak III – Tablica S7), što je vjerojatno posljedica ishrane ribom bogatom omega n-3 masnim kiselinama (Mourente i Tocher, 2009). Iako je važnost spomenutih esencijalnih masnih kiselina prepoznata u kontroliranoj reprodukciji komarče u akvakulturi gdje hrana bogata lipidima i omega-3 masnim kiselinama doprinosi kvalitetnijoj proizvodnji oocita i razvoju ličinki (Moretti i sur., 1999; Migaud i sur., 2013), moguća uloga u procesu promjene spola već kod dvogodišnjih populacija oko uzgajališta tuna još je nedovoljno istražena. Međutim, Simo-Mirabet i sur. (2018) zabilježili su da 15 % više uzgojnih komarči mijenja spol iz mužjaka u ženku pri hranidbi peletima u kojima je 60 % ribljeg ulja zamijenjeno s biljnim uljima. Spomenuti autori istakli su da udio i podrijetlo ulja u peletima utječe na promjenu spola, pogotovo na omjere gonadnih steroida, estradiol-17b (E2) i 11 ketotestesteron (11-KT), koji imaju ključnu ulogu u promjeni spola riba.

Reproduktivna plastičnost komarče također je uvjetovana i mnogim socijalnim čimbenicima jer je promjena spola kod dvospolaca povezana i s promjenama u načinu razmnožavanja, staništima i populacijskim gustoćama (Munday i sur., 2006, 2010; Avise, 2011; Erisman i sur., 2013). Stoga je veća gustoća komarči u okolici uzgajališta tuna možda doprinijela bržoj stopi pojavnosti ženki pri manjim dužinama. Primjerice, Ferreira i Russ (1995) zabilježili su smanjenje prosječne veličine protoginične kirnje (*Plectropomus leopardus*, Lacepède 1802) kao posljedicu intenzivnog ribolova, ali s odnosom spolova koji je ostao stabilan. Navedeni autori ukazali su na ranije mijenjanje spola ženke u mužjaka kao prilagodbu na veliki izlov kako bi se nadomjestilo smanjenje veličine u populaciji.

Smanjena ribolovna smrtnost omogućava obnovu starosne i veličinske strukture populacija vrsta u zajednicama riba (Robers i Polunin, 1991, 1992; Harmelin, 1999). Povećanje prosječne veličine te udjela velikih jedinki sve se više ističe kao jedan od ključnih pokazatelja učinkovite zaštite MPA-ova (eng. ***M**arine **P**rotected **A**reas*), jer ribolovom se najčešće izlovljavaju veće jedinke, a upravo one najviše doprinose reprodukcijskom potencijalu populacije (Pelletier i sur., 2005; Erzini i sur., 2006). Preporuke za mjere zaštite komarče na području RH, koje uključuju podizanje minimalne lovne veličine sa 20 na 30 cm te vraćanje lovostaja u vrijeme mriješćenja (Dulčić i Kovačić, 2020), već se provode unutar akvatorija uzgoja tuna zbog zabrane ribolova. Poistovjećenost uzgajališta tuna s ulogom i učincima MPA-ova (Šegvić-Bubić i sur., 2011b; Žužul i sur., 2019) stoga je potvrđena i ovim istraživanjem. Međutim, uzgojni lokaliteti kao takvi pružaju više od područja s ribolovnim ograničenjima i strukturalnom složenošću jer se stalnim unosom energetski bogate hrane pospješuje kondicijski i reprodukcijski potencijal kavezno-povezanih populacija.

Uloga uzgajališta tuna, energetski bogatog staništa, vrednovana je indeksom kondicije (Kn) koji odražava energetsko stanje jedinke preko dužinsko-masenog odnosa (Lloret i sur., 2014). Indeks kondicije je također uvjetovan starošću ribe, reproduktivnim statusom, godišnjim dobom, dostupnošću hrane te raznim drugim fiziološkim stanjima (Le Cren, 1951). Dodatno, promatran je i hepatosomatski indeks (HI) budući da je jetra organ za pohranu lipida kod većine pridnenih vrsta riba te ima važnu fiziološku ulogu u reprodukciji, prvenstveno kroz opskrbu energije za razvoj ovarija. Razvoj gonada promatran je gonadosomatskim indeksom (GSI) imajući na umu godišnje promjene u vrijednostima indeksa uvjetovane sazrijevanjem gonada, pripremu za mrijest i razdoblje nakon mrijesta (Lloret i sur., 2014).

U predmetnom istraživanju uočeno je da kavezno-povezane komarče i bukve imaju bolju kondiciju u odnosu na prirodne populacije na cjelogodišnjoj razini. Dostupnost i kvaliteta hrane smatraju se glavnim čimbenicima odgovornim za raspodjelu energije u različitim fiziološkim procesima kod riba (Adams i sur., 1982). Vrijednosti Kn indeksa kod prirodnih populacija kolebale su u ovisnosti o sezoni, što je pak povezano s intenzitetom prehrane, reproduktivnim ciklusom i temperaturom mora (Lambert i Dutil, 1997). Povećani indeks kondicije kavezno povezanih vrsta je bio je popraćen i većim vrijednostima GSI indeksa tijekom cijele godine u odnosu na divlje populacije, gdje su populacije komarči oko uzgajališta, uz znatno veće vrijednosti Kn indeksa u toplijem dijelu godine, imale niži HI indeks (Članak I – Slika 4). Spomenuto kolebanje indeksa upućuje na to da kavezno povezane komarče biraju strategiju povećanja reproduktivnog potencijala, naspram strategije promoviranja somatskog rasta i pohrane energije (Fernández-Palacios i sur., 2011). Osim toga, viši reproduktivni potencijal komarči u okolici kaveza tuna bio je izražen kroz povećane vrijednosti GSI tijekom razdoblja mrijesta kada je zabilježen pad u kondiciji (Članak I – Slika 4; Članak II – Slika 5). Međutim, dužinsko-maseni odnos kavezno-povezane komarče imao je istu stopu rasta mase po jedinici dužine kao i uzgojne komarče (Članak II – Tablica 3; Slika 4) uslijed sličnih trofičkih uvjeta opisanih sa stalnim unosom energetski bogate hrane u okoliš. No kontroliranija hranidba, kao i kasnija promjena spola (Članak II – Slika 6), vjerojatno su čimbenici koji su pogodovali boljem kondicijskom statusu uzgojne komarče naspram kavezno-povezane pri istim dužinama. Smanjenje stope rasta mase po jedinici dužine, pak, izraženije je kod divljih nego kavezno-povezanih komarči, što se poklapa s dužinama na kojima je zabilježen početak promjene spola prirodnih populacija. Naime, promjena spola kod protandričnih vrsta fiziološki je složen proces te je energetska podrška ključna za razvoj ovarija i kasnije zrelih oocita. U uvjetima niskog kondicijskog statusa ili nepovoljnih uvjeta okoliša jedinka može i prolongirati promjenu spola kako bi osigurala fiziološku homeostazu (Godwin, 1994). U odnosu na divlja ihtionaselja koja su stalno izložena okolišnim i ribolovnim pritiscima, što posljedično utječe na dostupnost njihovog plijena, kavezno povezana ihtionaselja imaju značajnu trofičku podršku koja osigurava bolji kondicijski i reproduktivni status te posljedično uspješan mrijest (Fernandez-Jover i sur., 2007; Arechavala-Lopez i sur., 2011; Dempster i sur., 2011; Fernandez-Jover i sur., 2011; Sanchez-Jerez i sur., 2011; Woodcock i sur., 2018; Gonzalez-Silvera i sur., 2020). Odljev jaja i ličinki izvan uzgojno-koncesijskih područja pospješuje lokalni ribolov (Sanchez-Jerez i sur., 2011), u skladu s učinkom zaštićenih morskih područja. Nedavnim istraživanjem populacijske genetike komarče u Jadranu u kombinaciji s Lagrangeovskim disperzijskim modelom, uvažavajući oceanografske uvjete u bazenu, uzgajališta tuna prepoznata su kao područja gdje se komarča mrijesti te je potvrđen odljev jaja i mlađi u priobalna rastilišta duž Jadrana (Žužul i sur. 2019). Stoga uzgajališta sinergijski s drugim okolišnim čimbenicima poput temperature mora, doprinose brojnosti prirodnih populacija komarče u Jadranu.

Spomenuti su autori također prepoznali da je divlja mlađ komarče uzorkovana u blizini kaveznih instalacija bijele ribe na otoku Braču mlađ uzgojno-atlantskog podrijetla, što upućuje na mogućnost spontanog mrijesta uzgojne ribe u kavezima. Ovakav obrazac već je prepoznat u Grčkoj (Dimitriou i sur., 2007; Somarakis i sur., 2013) gdje se povećanje brojnosti populacija komarče u obalnim područjima pripisuje zbjegovima putem oplođenih jaja iz kaveznih instalacija. Sličan trend prisutan je i duž istočne obale Jadrana gdje je paralelno s povećanjem ulova, zabilježen i značajan porast uzgojnih kapaciteta komarče, što pogoduje većem broju zbjegova koji doprinose povećanju ribolovnog ulova vrste. Molekularnim biljezima prepoznata je introgresija uzgojnog genotipa komarče u prirodnim staništima istočnog Jadrana (Žužul i sur., 2019). Iako su se molekularne metode pokazale kao jako učinkovit alat pri identifikaciji zbjegova komarče, njihova primjena iziskuje specifično znanje, vrijeme te veće financijske izdatke za otkrivanje podrijetla jedinke. Stoga razvoj brzog, jeftinog, a ujedno i preciznog alata temeljenog na morfološkim karakteristikama predstavlja sljedeći korak u raspoznavanju kako bi se omogućila bolja kontrola podrijetla lovine.

Fenotipska obilježja komarči divljeg i uzgojnog podrijetla. U predmetnom istraživanju, digitalne fotografije jedinki komarče korištene su za analizu i opis tjelesnih obilježja divljih, kavezno-povezanih i uzgojnih populacija u istočnom Jadranu. Primjena metode geometrijske morfometrije pokazala se učinkovitom u istraživanju s obzirom na uspješnost razlikovanja podrijetla jedinki s konfiguracijom od 18 homolognih anatomskih točki, uz vizualnu interpretaciju tjelesnih razlika. Pomoću kanoničke varijantne analize uočen je prosječan oblik komarči različitog podrijetla. Uzgojno je podrijetlo obilježeno zdepastijim oblikom tijela i zaokruženijim profilom glave sa supraterminalno pozicioniranim ustima, dok je divlje podrijetlo sadržavalo izduženiji tjelesni oblik te usta koja su subterminalno pozicionirana na većem profilu glave (Članak II – Slika 2).

Prepoznata tjelesna obilježja divlje i uzgojne komarče u istočnom Jadranu u skladu su s dosadašnjim nalazima (Arechavala-Lopez i sur., 2012a; Šegvić-Bubić i sur., 2014). Kod uzgojnih populacija stalna izloženost inertnoj i po strukturi mekanijoj peletiranoj hrani koja propada kroz vodeni stupac vjerojatno je uvjetovala razvoj manje glave u odnosu na veličinu glave divljih populacija, sa supraterminalnim pozicioniranim ustima. Predmetnim istraživanjem uočen je sličan obrazac prilagodbe glave kod populacija u okolici uzgajališta tuna gdje varijabilnost položaja usta i oblika glave predstavljaju odgovor plastičnosti na plijen sličnih karakteristika. Stoga je promjena oblika glave u ovisnosti o navikama hranjenja vrste zbog prilagodbe dostupnosti i ponašanju plijena različite veličine potvrđena ovim istraživanjem. U kontroliranim uvjetima Russo i sur. (2007) uočili su prilagodbu oblika tijela u odnosu na sastav plijena komarče tijekom različitih ontogenetskih stadija razvoja vrste, međutim, u predmetnom je istraživanju po prvi put u prirodnom okolišu uočena gore navedena tjelesna prilagodba. S obzirom na robusnost ulaznih podataka s višegodišnjim karakterom uzorkovanja, oblik uzgojnih populacija ili pak onih oko uzgajališta tuna, može se klasificirati kao tipičan uzgojni morfotip. S druge strane, zdepasti tjelesni oblik komarče popraćen je visokim indeksom kondicije i niskim gonadosomatskim indeksom. Sličan obrazac primijetili su i Grigorakis i sur. (2002) povezujući peritonealno i perivisceralno nakupljanjem lipida sa zaokruženim oblikom tijela komarči u uzgoju. Međutim, morfometrijske osobine riba podložne su promjenama u ovisnosti o ontogenetskom razvoju jedinke uključujući spolno sazrijevanje te različite okolišne uvjete (primarno temperaturu i hidrodinamične uvjete) (Koumoundouros i sur., 2001; Georgakopoulou i sur., 2007; Fisher-Rousseau i sur., 2010; Georga i Koumoundouros, 2010; Loizides i sur., 2014). U uzgojnim uvjetima, istražujući fenotipsku povezanost mlađi i odraslih jedinki komarče, Fragkoulis i sur. (2021) uočili su da značajne promjene u tjelesnom obliku ribe prestaju nastupanjem spolne zrelosti pri ukupnoj dužini od 20 cm, naglašavajući značajan stupanj nasljeđivanja pojedinih tjelesnih obilježja, poput visine tijela, što upućuje na to da zdepasti oblik komarče u uzgoju može dodatno biti uvjetovan aktivnostima selekcijskog programa.

U ovom je istraživanju sveukupna klasifikacijska točnost CVA analize jedinki prema pripadajućem podrijetlu iznosila 84,04 % (Članak II – Tablica 2). Međutim, parne usporedbe zabilježile su udio od 0,4 % pogrešno pridruženih jedinki divljeg i uzgojnog podrijetla, dok je udio za pogrešno pridružene jedinke divljeg i kavezno-povezanog podrijetla iznosio 21%. Budući da divlje i kavezno-povezane komarče obitavaju u staništima gdje prevladavaju različiti trofički resursi, provedena je dodatna analiza koja je uključivala uzorkovanje 19 točaka krivine kako bi se istražilo utječe li varijabilnost morfoloških obilježja na profilu glavu na uspješnost određivanja jedinki prema pripadajućem podrijetlu (Članak III – Slika S2). Klasifikacijska točnost CVA analize povećala se sa 77 % na 85 %, odnosno broj pogrešno klasificiranih se smanjio uključivanjem krivine glavenog profila u konfiguraciju homolognih anatomskih točaka. Dodatno kvantificiranje krivina tjelesnih struktura komarče polučilo je veći klasifikacijski uspjeh i preciznije određivanje jedinki prema podrijetlu**,** što inače ne bi bilo moguće isključivom primjenom homolognih anatomskih točaka.

Pokrivenost tijela s 18 lako uočljivih homolognih anatomskih točki predstavlja konfiguraciju koja jasno razlikuje divlju od uzgojne komarče, iako dodatno uzorkovanje točkama krivine može unaprijediti klasifikacijsku točnost i opis izgleda. Ovim istraživanjem poduzet je prvi korak prema automatskom uzorkovanju tjelesnog oblika za potrebe raspoznavanja podrijetla komarče jer je sveukupnim uzorkom od 1438 jedinki stvorena dovoljno velika baza podataka koja može poslužiti za treniranje algoritama strojnog učenja. No daljnje prikupljanje tjelesnih oblika osiguralo bi još kvalitetniju osnovu za preciznije strojno učenje automatiziranog uzorkovanja, čime bi se pospješio razvoj brzog alata za raspoznavanje podrijetla komarči duž istočnog Jadrana.

S obzirom na uzorak divlje (TL 25,7 ± 2,2 cm) i uzgojne (TL 27,7 ± 2,0 cm) komarče u predmetnom istraživanju, prepoznavanje zbjega u prirodi na osnovi samog oblika tijela moguće je, ali je primjenjivo samo kod bježanja odraslih jedinki. Dosadašnja istraživanja genetske raznolikosti identificirala su prisutnost uzgojnih genotipova u prirodnim populacijama lubina i komarče na području istočnog Jadrana, s prosječnim udjelima od 15 % (Šegvić-Bubić i sur., 2011a, 2017). Slična situacija zabilježena je u vodama Cipra (13-15 %, Brown i sur., 2015) i u zapadnom Sredozemnom moru (11-20 %), na temelju usporedbe ribljih ljusaka (Izquierdo-Gómez i sur., 2016). Međutim, interakcija s izvornom biocenozom može utjecati na fenotipske promjene uzgojnih jedinki jednom kad se nađu u prirodnim uvjetima, gdje uzgojno karakteristični morfotip može poprimiti obilježja divljeg morfotipa. Pri raspoznavanju podrijetla ribe treba uzeti u obzir da dinamika morfološke prilagodbe ovisi o tome u kojoj je fazi ontogenetskog razvoja došlo do bijega jer se postepeno smanjuje od ranih razvojnih prema odraslim stadijima. Tijekom poribljavanja lagune Papas u Grčkoj s mlađi komarče težine oko 5 g, Rogdakis i sur. (2011) prepoznali su vremenski raspon od šest do sedam mjeseci potreban da komarče iz uzgoja u ranim razvojnih stadijima poprime morfološka obilježja divljih jedinki zbog promjene okoliša i režima ishrane. Sličan obrazac prilagodbe zabilježili su i Toledo-Guedes i sur. (2021) istražujući lubina na Kanarskom otočju, gdje se uzgaja kao alohtona vrsta. Prebjezi lubina morfološki su više odgovarali divljem tipu, kao rezultat prilagodbe uzgojnog zdepastijeg tijela prema izduženijem obliku. Telemetrijskim praćenjem simulacije bijega odrasle uzgojne komarče iz priobalnog uzgajališta srednjeg Jadrana uočeno je zadržavanje i do 70 % označenih komarči u blizini kaveza tijekom prvih triju tjedana od bijega (Šegvić-Bubić i sur., 2018). Tijekom tog razdoblja, zbjegovi su provodili dnevno-noćne migracije tako da su noću migrirali u okolna područja, a tijekom dana boravili su oko uzgajališta hraneći se nepojedenom hranom (peletima) koja propada kroz kaveze, prilagođavajući se na novi okoliš. Unutar četiri mjeseca od zbjega iz uzgajališta većina je jedinki nepovratno napustila uzgojno područje. Uzevši u obzir opisani obrazac ponašanja prebjega koji se također poklapa s iskustvima uzgajivača, upotreba tjelesnih obilježja divlje i uzgojne komarče u svrhu razlikovanja podrijetla vjerojatno je najučinkovitija u spomenutom vremenskom razdoblju. Uvažavajući rezultate istraživanja Fragkoulisa i sur. (2021) koji upućuju na izostanak značajnih fenotipskih promjena komarče nastupanjem spolne zrelosti, potrebna su ipak daljnja istraživanja fenotipskog odgovora zbjegova odraslog stadija tijekom dužeg perioda obitavanja u prirodnim staništima.

Uz vanjska tjelesna obilježja, u predmetnom istraživanju promatrana je obojenost tijela pomoću digitalnih fotografija jedinki. Ovakav metodološki pristup je prvi put u ovom istraživanju primijenjen na ribama gdje je kvantifikacija doprinosa određene boje po tjelesnoj površini omogućila vizualno razlikovanje podrijetla komarče. Srebrnkasto-siva obojenost izdvojena je kao dominantna jer pokriva glavninu tjelesne površine vrste bez obzira na podrijetlo jedinke. Uzgojna komarča ima veću tjelesnu pokrivenost tamnim i blijeđim nijansama srebrnkasto-sive boje u usporedbi s divljim i kavezno-povezanim jedinkama osobito na glavi, leđnom dijelu tijela i perajama (Članak III – Slika 6). Potvrđeni su dosadašnji kvalitativni opisi obojenosti komarče gdje su jarkije boje izražajnije kod divljih u odnosu na uzgojne jedinke (Grigorakis i sur., 2002; Rogdakis i sur., 2011; Šimat i sur., 2012). Visoka gustoća nasada, stalna izloženost suncu i niski sadržaj prirodnih pigmenata u ribljoj hrani predstavljaju glavne čimbenike koji doprinose prekomjernoj proizvodnji melanina, odnosno pigmenta koji znatno doprinosi pojavnosti tamnije obojenosti kod kavezno uzgojene ribe (Pavlidis i sur., 2011). U uniformnim uzgojnim uvjetima jedinke pokazuju veću međusobnu ujednačenost u obojenosti u odnosu na druga dva podrijetla koja slobodno obitavaju u prirodnim staništima (Članak III – Tablica 2, Slike 4 i S5). Međutim, zbog izmijenjenog ponašanja u odabiru plijena gdje kavezno-povezane jedinke aktivno biraju ribu naspram drugog plijena, sadržaj karotenoida potreban za ispoljavane jarkijih boja nije postignut ishranom. Unatoč tome što dijele sličnosti u nijansama srebrnkasto-sive boje s divljim jedinkama (Članak III – Slike 3, 4), komarče pod utjecajem uzgoja tuna pokazale su veću sličnost u obojenosti prema tamnijim i blijeđim uzgojnim jedinkama (Članak III – Tablica 2).

Uz tjelesna obilježja i obojenost populacija komarči različitog podrijetla, oblik otolita također je bio predmetom istraživanja. Otoliti su strukture smještene u šupljini unutrašnjeg uha i služe kao organ za ravnotežu i doprinose osjetilu sluha. Tradicionalno se koriste u ribarstvenoj biologiji s ciljem klasifikacije stokova, određivanja dobi ili veličine ribe (Afanasyev i sur., 2017). Odnedavno, s porastom akvakulture i prisutnosti prebjega u okolišu, proučavan je oblik otolita riba divljeg i uzgojnog podrijetla, te se oblik pokazao kao informativni fenotipski biljeg za raspoznavanje podrijetla populacija komarči (Arechavala-Lopez i sur., 2013). Analizom varijabilnosti obrisa otolita populacija komarči različitog podrijetla zapažen je urez u anteriorno-ventralnom području otolita (Članak III – Slika 7) koji predstavlja glavno razlikovno obilježje uzgojnih i divljih jedinki. Međutim, veća varijabilnost veličine ureza otolita zabilježena je u uzgojnim u odnosu na divlje populacije te su prepoznata tri tipa uzgojnog oblika otolita zbog prisustva različitih vršnih struktura na anteriornom području (Članak III – Slika 6, S7). Slične razlike u obrisu otolita divlje i uzgojne komarče uočili su i Geladakis i sur. (2020) u Grčkoj, što upućuje da okolišni čimbenici poput izmijenjene ishrane imaju značajnu ulogu u oblikovanju uzgojno specifičnog oblika otolita, dok je važnost genotipa prisutna u manjoj mjeri. Uspješnost klasifikacije LDA analize iznosila je 87 % za uzgojne te 71 % za divlje jedinke (Članak III – Tablica 4). Klasifikacijski rezultati podupiru tezu o odvojenim stokovima uzgojnih i divljih jedinki, budući da zadovoljavaju kriterij klasifikacije (>70 %) na temelju kojeg se riblje populacije smatraju odvojenim stokovima (Harbitz i Albert, 2015).

Ovim su istraživanjem utvrđene razlike i u kemijskom sastavu tkiva. Odnos postotnog udjela ukupne masti i sadržaja vode u kemijskom sastavu mesa uzgojne komarče iznosio je 10,2 % i 70,5 %, dok su se vrijednosti kod divljeg i kavezno-povezanog uzorka kretale u prosjeku oko 1,65 % i 78,5 %. Grigorakis (2007) je zabilježio da prosječna vrijednost masti u divljim populacijama komarči iznosi 1,4 % dok su se kod riba sa uzgajališta vrijednosti kolebale i do 20,4 %. Popović i sur. (2012) ustanovili su prisutnost zbjegova u lovini komarči na području srednjeg Jadrana primjenom kemijske analize tkiva, gdje su unutar lovine uočili dvije skupine komarči s različitim sadržajem udjela masti od 1,59 % i 10,4 %. Stoga se sadržaj masti u kemijskom sastavu tkiva komarče može dodatno koristiti kao alat za prepoznavanje zbjega, jer ribe u uzgoju imaju povećani udio masti u odnosu na udio bjelančevina (Arechavala-Lopez i sur., 2013; Tičina i sur., 2020). Također, predmetnim istraživanjem uočene su i povećane razine oleinske (18:1n9c) i linolne (18:2n6c) kiseline kao i niski omjer n-3 i omega n-6 masnih kiselina kod uzgojnih komarči naspram drugih dvaju promatranih podrijetala što potvrđuje njihovu korisnost u prepoznavanju prehrane ribljim peletima bogatim uljima biljnog podrijetla. Iako je određivanje udjela masnih kiselina kopnenog podrijetla te sadržaja masti kod morskih organizama metodološki provediv postupak, njihova primjena u raspoznavanju podrijetla ograničena je na prepoznavanje nedavno prebjegle ribe, jer se kemijski trag u tkivu brzo gubi u trenutku kada se uzgojna komarča prilagodi novim trofičkim resursima u prirodnim staništima (Arechavala-Lopez i sur., 2013).

ZAKLJUČCI

Na osnovu provedenih istraživanja, gdje su se vrednovali različiti utjecaji uzgajališta tuna na fenotipska obilježja lokalnih populacija komarči te fenotipska obilježja komarči divljeg i uzgojnog podrijetla, doneseni su sljedeći zaključci:

1. Antropogeno uvjetovani okoliši u istočnom Jadranu, poput uzgajališta tuna, mogu djelovati kao veliki umjetni FAD-ovi (engl. *fish aggregating devices*) koji okupljaju priobalne riblje vrste tijekom cijele godine. Oko istraživanih kaveza tuna zabilježene su dvadeset i četiri vrste riba, uglavnom iz porodica Sparidae, Carangidae i Serranidae. Ukupna brojnost sastava ihtionaselja na uzgajalištima bila je značajno veća od one utvrđene na kontrolnim lokacijama. Bukva i komarča bile su najčešće zastupljene vrste.
2. Na istraživanim uzgajalištima tuna značajna varijabilnost brojnosti i bogatstva vrsta uočena je u odnosu na dubinu, dok je varijabilnost između godišnjih doba manje izražena. Najveća brojnost i bogatstvo vrsta uočeni su ispod uzgajališta pri morskom dnu, dok daljnji prostorni utjecaj privlačenja ne prati gradijent udaljenosti već pokazuje opadanje samo nekoliko stotina metara od uzgajališta.
3. Trajna prostorna i vremenska zastupljenost komarči oko uzgajališta tuna upućuje na važnu ulogu jadranskih uzgajališta tuna kao pogodnih staništa za divlju komarču. Analizom sadržaja želudaca komarči potvrđena je prisutnost ribe kao dominantnog plijena (%IRI=92), što podupire hipotezu da se populacije u okolici uzgajališta tuna hrane nepojedenim ostacima male plave ribe.
4. Izmijenjeni okolišni uvjeti u akvatoriju uzgajališta tuna, uključujući unos hrane za tune te bržu dinamiku izmjene vodenih masa zbog odabira lokacije uzgoja, uvjetovali su specifičnu morfološku prilagodbu komarče odnosno ispoljavanje kavezno povezanog morfotipa. U odnosu na divlje populacije komarče u okolici kaveza tuna imaju manju i tuplju glavu, vretenastije tijelo i blijeđu srebrenkasto-sivu obojenost.
5. Rezultati modela odabira plijena u odnosu na ukupnu tjelesnu dužinu upućuju na to da komarča selektivno odabire trofičke resurse koji doprinose kvalitetnijoj proizvodnji oocita i razvoju ranih razvojnih stadija.

6. Promjena spola od mužjaka prema ženki pri manjim ukupnim tjelesnim dužinama izraženija je kod dvogodišnjih i trogodišnjih komarči oko uzgajališta tuna naspram onih iz prirodnih staništa tijekom razdoblja mrijesta. Kavezno povezane populacije imaju značajnu trofičku podršku koja osigurava bolji kondicijski i reproduktivni status te posljedično biraju strategiju povećanja reproduktivnog potencijala naspram somatskom rastu i pohrani energije.
7. Razlikovna fenotipska obilježja divljih i uzgojnih komarči najviše se očituju u vanjskim tjelesnim obilježjima. Uzgojne komarče imaju zdepast oblik tijela sa zaokruženijim profilom glave i supraterminalnim pozicioniranim ustima, dok je izduženije tijelo sa subterminalnim ustima na većem profilu glave svojstveno jedinkama divljeg podrijetla. U usporedbi s populacijama koje obitavaju u prirodnim i kavezno povezanim staništima uzgajališta tuna, komarče u uzgoju imaju veću tjelesnu pokrivenost s tamnim i blijeđim nijansama srebrnkasto-sive boje na glavi, leđnom dijelu tijela i perajama.
8. Primjena metode geometrijske morfometrije na relevantnim fenotipskim biljezima (oblik tijela i otolit) pokazala se uspješnom u razlučivanju divlje od uzgojne komarče. Konfiguracija od 18 homolognih anatomskih točki na oblik tijela dostatna je za razlikovanje podrijetla komarče. Analizom obrisa otolita zapaženo je da urez u anteriorno-ventralnom području otolita predstavlja glavno razlikovno obilježje na temelju kojeg je ostvarena klasifikacijska uspješnost veća od 70 %. Iako je na osnovi oblika tijela postignuta visoka klasifikacijska točnost jedinki prema pripadajućem podrijetlu od 99,6 %, otolit se pokazao funkcionalnijim biljegom zbog biomineralne strukture i postojanog oblika u odnosu na okolišno plastičnija tjelesna obilježja komarče.
9. Potrebna su daljnja biološka i ekološka istraživanja komarče, uključujući i druge riblje vrste od važnosti za ribarstvo i akvakulturu, kako bi se stekle spoznaje o fenotipskim promjenama tijekom razvoja vrste u prirodnim i antropogeno uvjetovanim staništima istočnog Jadrana. Kontinuirano prikupljanje podataka fenotipskih obilježja vrste omogućilo bi razvoj učinkovitijeg alata za prepoznavanje prebjega kroz primjenu strojnog učenja, ali i pružilo značajnu podršku selekcijskim programima na ciljana obilježja, poput stope rasta i tjelesnog oblika.

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ŽIVOTOPIS

Igor Talijančić rođen je 17. listopada 1988. godine u Splitu, gdje je završio V. gimnaziju (opću). Akademske godine 2007./2008. upisao je preddiplomski studij Sveučilišnog studijskog centra za studije mora Sveučilišta u Splitu, smjer Morsko ribarstvo, te na istoimenom diplomskom studiju 2013. godine stekao sveučilišni stupanj magistra inženjera. Zaposlio se na mjestu stručnog suradnika u Institutu za oceanografiju i ribarstvo u Splitu 2015., gdje je obavljao poslove povezane s akvakulturom, terenskim istraživanjima te prikupljanjem, obradom i analizom podataka. Od kolovoza 2018. doktorand je na projektu "Utjecaj akvakulture na prirodne morske populacije (Aquapop)", čija je voditeljica dr. sc. Tanja Šegvić Bubić. Upisao je poslijediplomski doktorski studij Oceanologije na Geološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu 2016. godine pod mentorstvom dr. sc. Tanje Šegvić Bubić, više znanstvene suradnice pri Institutu za oceanografiju i ribarstvo u Splitu. Prvi je autor na dvama obavljenim znanstvenim radovima te koautor u jedanaest znanstvenih radova. Sudjelovao je na međunarodnim znanstvenim konferencijama kao prvi autor s trima usmenim prezentacijama i četirima poster prezentacijama te kao koautor na devet prezentacija. Održao je ciklus znanstveno-popularnih predavanja pod nazivom "Čija si ti mala – Priča o podrijetlu komarče iz Jadrana" u Splitu i Zadru. Dobitnik je stipendije "Euromarine Individual Fellowship Programme 2018."

Sudjelovao je na šest međunarodnih i nacionalnih usavršavanja povezanih s geometrijskom morfometrijom u 2D-u i 3D-u te u analizi podataka u programskom jeziku R:

- Institut za oceanografiju i ribarstvo (Split, Hrvatska): 11. srpnja 2021. "An overview of otolith microchemistry: an introduction into some of the analytical challenges and statistical approaches";
- Slicermorph projekt (online tečaj): Od 17. kolovoza do 21. kolovoza 2020. "3D morphometrics and image analysis workshop";
- Physalia Courses (Berlin, Njemačka): Od 5. ožujka do 9. ožujka 2018. „Geometric morphometrics";
- Transmitting science (Barcelona, Španjolska): Od 19. lipnja do 22. lipnja 2018. "Integration and modularity with geometric morphometrics";
- Transmitting science (Barcelona, Španjolska): Od 18. siječnja do 22. siječnja 2016. "Geometric morphometrics in R";

- FAO-ADRIAMED project (Rim, Italija): Od 7. rujna do 11. rujna 2015. „The use of the R programing language for statistical analysis“.

ZNANSTVENA AKTIVNOST I PUBLIKACIJE

CROSBI PROFILE: Igor Talijančić (CROSBI Profil: 35578, MBZ: 375304)

ZNANSTVENI RADOVI

Talijančić, I., Žužul, I., Kiridžija, V., Šiljić, J., Pleadin, J., Grubišić, L., Šegvić-Bubić, T. (2021) Plastic responses of gilthead seabream *Sparus aurata* to wild and aquaculture pressured environments. *Frontiers in marine science*, 8, 694627.

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SUDJELOVANJE NA PROJEKTIMA

ADRION Interreg ARIEL: Promoting small scale fisheries and aquaculture in Adriatic-Ionian macro region, suradnik projekta.

Hrvatski savez za sportski ribolov na moru. Biheviorizam i fenotipska obilježja prebjega (BI-FEN) iz akvakulture kod vrsta od interesa za športsko-rekreacijski ribolov duž istočne obale Jadrana, suradnik projekta.

Hrvatski savez za sportski ribolov na moru. Biološki odgovor plavoperajne tune (*Thunnus thynnus*) na sportsko rekreacijski ribolov metodom ulovi i pusti, suradnik projekta.

Hrvatski savez za sportski ribolov na moru. Određivanje genetske strukture izvornih prirodnih populacija vrsta od interesa za športsko - rekreacijski ribolov i marikulturuduž istočne obale Jadrana (gof, zubatac, lubina i komarča), suradnik projekta.

HRZZ Istraživački projekt "Utjecaj akvakulture na prirodne morske populacije", suradnik projekta.

UKF Istraživački projekt - Natjecanje između autohtone *Ostrea edulis* i invazivne *Crassostrea gigas* školjke, suradnik projekta.

Uprava ribarstva: DCF - Nacionalni program za prikupljanje podataka o ribarstvu, suradnik projekta.

Uprava ribarstva: IPT - Utvrđivanje indeksa prirasta tuna u kavezima, suradnik projekta.

Uprava ribarstva: PRUT - Praćenje ribolova i uzgoja tuna, suradnik projekta.

Uprava ribarstva: SCT - Primjena sustava stereoskopske kamere u procjeni tuna namijenjenih kaveznom uzgoju, suradnik projekta.