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Spawning segregation and philopatry are major prezygotic barriers in sympatric cryptic *Mugil cephalus* species

Ségrégation sur les zones de ponte et philopatrie sont les principales barrières pré-zygotiques chez les espèces sympatriques du complexe d'espèces Mugil cephalus

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ABSTRACT

The flathead mullet, *Mugil cephalus*, is a commercially vital fish in fisheries and aquaculture worldwide. Genetic analyses have recently revealed three cryptic species of *M. cephalus* in the Northwest Pacific. These species are sympatric in Taiwanese waters and specific reproductive behaviors have been suggested to be a major prezygotic barrier. Species composition was evaluated in samples of *M. cephalus* at different growth stages collected from various habitats (offshore spawning ground, estuarine nursery and feeding areas) over several months or years. The gonadosomatic index of adults and the body length of juveniles were recorded to determine the reproductive season and recruitment periods in estuaries. The results revealed partially temporal spawning isolation between species pairs, spatial segregation on specific spawning grounds and strong philopatry preclude hybridization. Thus, the results imply that traditional fisheries of mature fish in the Taiwan Strait target only one species, whereas aquaculture in Taiwan contain juveniles of all three species collected in estuaries. The ecological niche and demography of these species must be investigated further to estimate the impact of juvenile sources on aquaculture.

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R É S U M É

Le mullet à grosse tête, *Mugil cephalus*, est une ressource halieutique essentielle dans de nombreux pays, en particulier à Taïwan. Récemment, des analyses de génétique des populations ont révélé l'existence, dans le Pacifique nord-ouest, de trois espèces cryptiques au sein de cette espèce nominale. Ces espèces sont sympatriques dans les eaux taïwanaises et leur isolement génétique pourrait être lié à des comportements reproducteurs spécifiques. Afin de tester cette hypothèse, la dynamique du recrutement et

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l'évolution spatio-temporelle de l'indice gonadosomatique ont été étudiées dans trois estuaires (au Nord et au Sud de Taiwan) et un des sites de ponte. Un total de 813 individus a été analysé, l'espèce de chacun d'eux ayant été identifiée grâce au polymorphisme de séquence du cytochrome oxydase 1. La période de reproduction de ces espèces a ainsi été précisée, de même que leur comportement reproducteur. Il a été ainsi montré que la zone de ponte située dans le détroit de Taïwan, et faisant l'objet d'une pêche traditionnelle, n'est utilisée que par une seule des espèces. L'isolement reproducteur de ce complexe d'espèce cryptique serait le résultat d'une maturation sexuelle partiellement asynchrone et surtout d'une ségrégation spatiale au moment de la reproduction. Le recrutement des juvéniles des trois espèces dans les estuaires pose la question de l'impact de la pêche destinée à alimenter les piscicultures taïwanaises sur la dynamique des populations de ces espèces.

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1. Introduction

Species boundaries remain a controversial topic when the definition of species is limited to a method rather than to a set of properties [1]. Among the properties generally advocated, reproductive isolation is probably the most popular because a species is essentially an independent evolutionary lineage. Although an increasing number of population genetics studies have demonstrated the presence of cryptic species in well-known nominal species [2–4], the evolutionary processes responsible for the speciation and maintenance of specific genetic integrity are generally only assumed. Allopatric speciation and physical barriers to gene flow can easily be proposed when cryptic species exhibit disconnected distribution ranges; by contrast, species with parapatric, overlapping, or sympatric ranges must have a more complex evolutionary history. This is often the scenario for marine species, among which divergences along ecological boundaries are thought to occur more frequently than they do among terrestrial organisms [5]. The genetic integrity of marine species is maintained through physiological or behavioral mechanisms that prevent gene flow and hybridization. Specifically, differences in spawning time, reproductive migration, ecological niche, mate recognition, environmental tolerance, and gamete compatibility may be involved in the evolution of marine species [6].

The flathead mullet, *Mugil cephalus* Linnaeus 1758, is found in coastal waters, lagoons, bays, and estuaries worldwide between the latitudes of 42°N and 42°S [7]. Recent population genetics studies have indicated that this nominal species is a complex of cryptic species [8–10]. Although most of the cryptic species exhibit allopatric distributions consistent with biogeographic barriers in the Atlantic and Indian Oceans (ocean, continent, thermal fronts), some species in the West Pacific exhibit overlapping distributions [8–14]. In the Northwest Pacific (NWP), a previous study [8] demonstrated that as many as three cryptic species, namely NWP1, NWP2, and NWP3 (corresponding to *Mugil* sp. C, *Mugil* sp. I, and *Mugil* sp. L, respectively, which were provisorily designated according to mitochondrial phylogeny of a global Mugilidae collection [10]), are sympatrically present in Taiwanese, Chinese, and Japanese estuaries. Although species boundaries have been inferred genetically, the mechanisms responsible for gene pool integrity are poorly understood, suggesting that specific life history traits are responsible for the genetic

isolation of these species; the gene flow may be limited or even prevented through specific reproductive behaviors that maintain the spatial and temporal isolation of these species during the spawning season. The various migratory behaviors in NW Pacific *M. cephalus* have been described, and the annual patterns in landings of mature fish as well as the recruitment of juveniles in estuaries has prompted suggestions that two populations characterized by specific life history traits exist in Taiwan [15–21]. Some fish may engage in annual reproductive migrations over large geographic areas from the Japan and Yellow Seas to the Taiwan Strait, where they spawn from mid-December to mid-January [15–17,22], whereas other fish reside in Taiwanese waters, where their juveniles recruit earlier (November) [19,23]. Large-scale migrations may be due to sea-surface temperature decreases that occur along the northern Chinese shoreline and in the Japan Sea when the cold China Coastal Current (CCC) is active in winter [17]. According to a satellite fishing ground data system (the NOAA-APT receiving system), in December, the CCC reaches the midwestern coast of Taiwan, where it forms a thermal front with the warm branch of the Kuroshio Current. This thermal front may stop the migration of mature adults [24]. In addition, an observation of numerous 5–15 mm *M. cephalus* larvae offshore the Kaoping River in January 1984 and 1985 suggested that the spawning ground is located southwest of Taiwan, near the thermal front [16,23]. Last, the single peak observed in the size distribution of oocytes suggested that the fish spawn once per year [16].

Whether fisheries influence spawning migration in the Taiwan Strait has not been established; thus, links between the three cryptic species and the observed reproductive strategies remain largely assumed [8]. This question must be answered to clarify the evolutionary processes responsible for the diversification of marine organisms and enable sustainable resource management in fisheries and aquaculture. *Mugil cephalus* landings in the NW Pacific currently indicate dramatic overexploitation (from 1980 to 2009, *M. cephalus* landings decreased from 6865 to 260 metric tons; Taiwan Fisheries Agency), which has boosted the potential development of mullet aquaculture in Taiwan and China. This changing pattern of fish exploitation may have dramatic consequences for the species complex. Traditional fisheries targeted only mature adults on the spawning grounds for only one month; however, for aquaculture purposes, juveniles are currently also targeted

for four months in the main Taiwanese estuaries, where mullets recruit. Thus, knowledge of the reproductive behavior and estuarine recruitment of these cryptic species is urgently required.

Therefore, in this study, species of *M. cephalus*, including mature adults, estuarine recruiting juveniles, and prespawning adults were collected at various locations and genetically identified, respectively. In addition, the gonadosomatic indexes (GSIs) of the collected fish were simultaneously recorded to reveal the existence of prezygotic barriers that prevent hybridization, such as ecological niche differences and temporal or spatial isolation. An approximation of the species compositions among juvenile recruits in estuaries, which are targeted by the aquaculture sector in Taiwan, was provided.

2. Materials and methods

2.1. Sampling design and biological measurement

To characterize the spawning activities of the three *M. cephalus* cryptic species that reside around Taiwan, three sampling schedules that covered the different life stages of fish from the spawning ground, feeding ground, and nursery ground were applied (Fig. 1). Spawning adults were sampled using purse seine nets during three consecutive years (2005–2007) [8] off the southwest coast of Taiwan near Kaohsiung, an *M. cephalus* spawning ground exploited by fishermen since the seventeenth century [15,23]. Adults of various sizes present at a feeding area were collected from the Kaoping River Estuary, the broadest tropical drainage in Taiwan (Fig. 1). Sampling was conducted monthly between August 2008 and March 2009, a period consisting of the months preceding and following the known spawning period of the fish. Juveniles from the Gonshitian Creek Estuary in Northern Taiwan and the Baoli Creek Estuary in Southern Taiwan (Fig. 1) were collected monthly from December 2011 to March 2012 by using a set net with the mesh size of 1 mm in areas of shallow water that enabled easily sampling. The recruit-

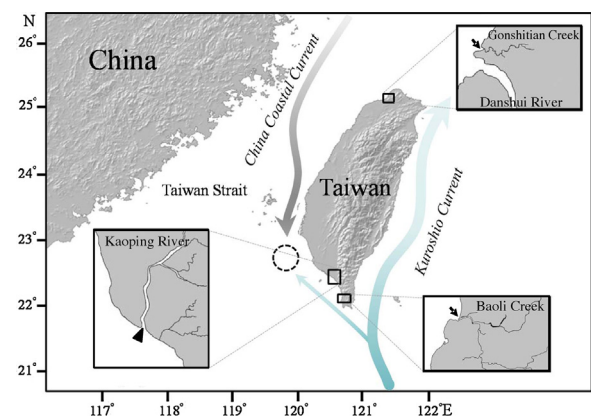


Fig. 1. (Color online.) Sampling location of fish in the traditional spawning ground offshore (dashed circle) from Kaohsiung, the nursery areas (arrows) of the Gonshitian and Baoli Creek Estuaries, and the feeding area (solid triangle) of the Kaoping River Estuary.

ment of *M. cephalus* juveniles in estuaries along the western coast of Taiwan that are known nursery areas of the fish was observed mainly in these four months [15,18]. In this study, feeding and nursery areas were distinguished in consideration of the size of the inhabited system of fish; a feeding area is also a nursery area because of the simultaneous presence of juveniles and adults, whereas only juveniles are observed in nursery areas.

The fork length, total weight (TW), and gonad weight (GW) of adult fish were measured. The total length of the juveniles was measured because the caudal fin in such fish was undeveloped. The GSI was calculated using the following equation: $100 \text{ GW}/(\text{TW} - \text{GW})$ [25]. The differences in mean values were tested using either the *t*-test or an analysis of variance (ANOVA). For each fish, muscle tissues were removed from the epi-axial region under the second dorsal-fin base and preserved in 95% ethanol.

2.2. DNA extraction and rapid screening

Genomic DNA was extracted from muscle tissue by using a DNA Purification Kit (Bioman, Taipei, Taiwan), preserved in a Tris EDTA (TE) buffer, and then quantified and diluted to $1 \text{ ng}/\mu\text{L}$ for use in polymerase chain reaction (PCR).

A rapid screening method that involved detecting the three cryptic species of *M. cephalus* in the NW Pacific by using a multiplex Cytochrome Oxidase I (COI) haplotype-specific PCR (MHS-PCR) was developed in a previous study [8] according to the recommendations in related studies [26,27]. Table 1 lists the primers used for rapid screening. PCR was performed using a Biometra TGradient Thermocycler with a $15\text{-}\mu\text{L}$ reaction volume containing $0.2 \mu\text{M}$ of dNTP, $1.5 \mu\text{L}$ of a $10 \times$ PCR buffer (Bioman), $0.5 \mu\text{M}$ of forward and reverse primers, 0.2 U of Taq DNA polymerase (Bioman), and $1.0 \mu\text{L}$ of template DNA. Mitochondrial DNA amplification was conducted using the following PCR program: 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 30 s after heating at 94°C for 5 min. The PCR products were electrophoresed in a 2.0% agarose gel (Bioman) and stained with ethidium bromide to characterize the size of bands by using ultraviolet transillumination. The PCR products of NWP1, NWP2, and NWP3 were 362 bp, 283 bp, and 549 bp, respectively, according to the COI sequences (Fig. 2).

3. Results

3.1. Spatial and temporal distribution in key habitats

Among the 151 spawning adults sampled from the spawning ground off the southwest coast of Taiwan in

Table 1
Primers used for rapid screening of three *Mugil cephalus* cryptic species.

Primer name	Primer Sequence (5' to 3')
NWP 1,2F	5' GCTTTTCCCGAATAAAAT 3'
NWP 3F	5' TAGTGCCTAAGCCTACTC 3'
NWP 1,3R	5' CGATCTGTTAGGAGTATGG 3'
NWP 2R	5' CTCATACGAAAAGGGGTGT 3'

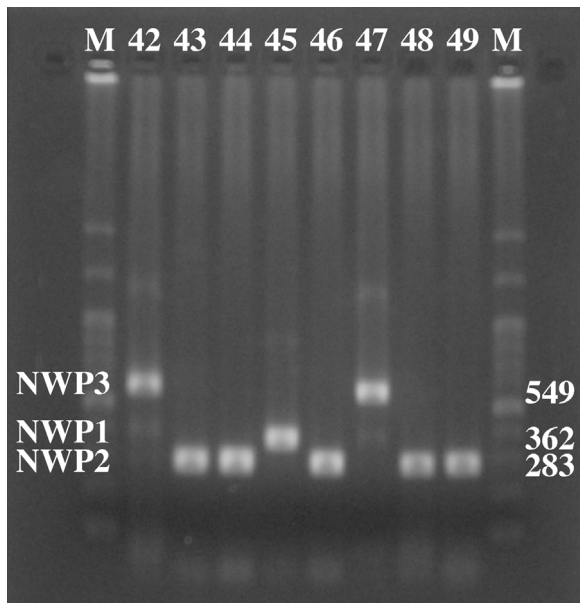


Fig. 2. PCR rapid screening of the three cryptic species of fish. M: 100 bp DNA ladder. No. 45: NWP1; No. 43, 44, 46, 48, and 49: NWP2; No. 42 and 47: NWP3. NWP: NW Pacific cluster.

December 2005, 2006, and 2007, only one species (NWP1) was represented (Table 2). By contrast, NWP1 accounted for only 1.8% of the 400 adults collected at the feeding ground of the Kaoping River Estuary, where the two other cryptic species (NWP2 and NWP3) were dominant, representing 46.0% and 52.8% of the species composition, respectively. Nevertheless, despite the low composition, NWP1 was observed in most monthly samples. From August to March, NWP3 was common, whereas the composition of NWP2 decreased in November and December (8.3% and 2.6%, respectively).

In the Gonshitian Creek Estuary in Northern Taiwan, no fish belonging to NWP3 were observed among juveniles

from December to March. Although NWP2 was the most abundant species among juveniles at this location (75% of the samples), in March, a clear shift occurred in NWP1, which accounted for all juveniles collected that month.

In the Baoli Creek Estuary in Southern Taiwan from December to March, no fish belonging to NWP1 were observed among the juveniles, whereas NWP2 (51.9–95.8%) and NWP3 (4.2–48.2%) were abundant. The composition of these two species exhibited an inverse trend compared with that of the samples collected from the Kaoping River. NWP2 was abundant in all four months, whereas the composition of NWP3 fluctuated widely.

3.2. Reproductive activity

The mean length of NWP1 on spawning and feeding grounds ranged from 526.0 ± 38.1 mm (in 2007) to 475.7 ± 34.6 mm (in 2005), and significantly exceeded the mean length of NWP2 (412.4 ± 39.3 mm) and NWP3 (392.2 ± 44.6 mm) on feeding grounds (ANOVA, $P < 0.05$; Table 3). The GSI of NWP1 on the spawning ground ranged from 17.0 to 24.6, exceeding the GSI of all NWP2 and most NWP1 and NWP3 on the feeding ground (>0.1 –20.1).

Species in the Kaoping River Estuary exhibited various reproductive characteristics (Fig. 3). Among seven large NWP1 fish, only two, measuring more than 480 mm, were mature (GSI > 17) (Fig. 3a). By contrast, although sexual maturation began at shorter lengths in NWP2 than in NWP1, no NWP2 collected from August to March exhibited a GSI exceeding 10 (Fig. 3b). Full sexual maturity (GSI > 15) was observed in NWP3 at lengths as short as 350 mm (Fig. 3c).

The spawning activity inferred according to temporal changes in GSIs revealed different patterns among the three species (Fig. 4). According to the limited quantity of data, the GSIs of NWP1 peaked at 20.1 in December (Fig. 4a). The GSIs of NWP3 increased gradually from October (7.2), peaked in November (17.9) and December (19.4), and remained relatively high until March (11.9–14.8; Fig. 4c). By

Table 2
Sampling information, size (n), and composition of three *Mugil cephalus* cryptic species.

Sampling schedule	Life stage	Location	Month/Year	n	NWP1 (%)	NWP2 (%)	NWP3 (%)
Spawning ground	Adult	Kaohsiung offshore	December/2005	44	100.0	0.0	0.0
			December/2006	50	100.0	0.0	0.0
			December/2007	57	100.0	0.0	0.0
Feeding ground	Adult	Kaoping River Estuary	August/2008	54	1.9	59.3	38.9
			September/2008	91	1.1	50.5	48.4
			October/2008	105	1.0	77.1	21.9
			November/2008	74	0.0	8.1	91.9
			December/2008	39	5.1	2.6	92.3
			February/2009	18	5.6	44.4	50.0
			March/2009	21	4.8	47.6	47.6
Nursery ground	Juvenile	Gonshitian Creek Estuary (North)	December/2011	24	0.0	100.0	0.0
			January/2012	24	0.0	100.0	0.0
			February/2012	24	0.0	100.0	0.0
			March/2012	24	100.0	0.0	0.0
			December/2011	54	0.0	61.1	38.9
Nursery ground	Juvenile	Baoli Creek Estuary (South)	January/2012	56	0.0	92.9	7.1
			February/2012	54	0.0	51.9	48.2
			March/2012	48	0.0	95.8	4.2

NWP: Northwest Pacific cluster.

Table 3

Sampling information, size (n), and ranges in fork length (FL), body weight (BW) and gonadosomatic index (GSI) of three *Mugil cephalus* cryptic species.

Location	Year	Species	n	FL (mm)	BW (g)	GSI
Kaohsiung offshore (Spawning ground)	2005	NWP1	44	423.0–555.0	850.0–1911.0	20.0–21.4
	2006	NWP1	50	421.4–533.7	962.0–1629.0	17.2–24.6
	2007	NWP1	57	459.6–623.9	856.0–1709.0	17.0–19.1
Kaoping River Estuary (Feeding ground)	2008–2009	NWP1	7	413.0–530.0	836.6–1656.0	>0.1–20.1
	2008–2009	NWP2	184	305.4–520.0	363.0–1699.2	>0.1–10.1
	2008–2009	NWP3	209	299.0–564.2	361.4–2108.6	>0.1–19.4

NWP: Northwest Pacific cluster.

contrast, the GSIs of NWP2 increased earlier, to approximately 10 in September, remained at that level between October and November, and then decreased to 3.9 in December (Fig. 4b).

3.3. Recruitment dynamic

The lengths of juveniles sampled from the Gonshitian Creek Estuary (north) and the Baoli Creek Estuary (south) of Taiwan from December 2011 to March 2012 ranged from 22.0 to 63.0 mm (Fig. 5). Recruitment of NWP2 juveniles occurred in both Northern and Southern Taiwan, whereas that of NWP1 and NWP3 juveniles was in Northern and Southern Taiwan, respectively.

In the north, juveniles collected from December to March exhibited lengths ranging from 22.0 to 33.5 mm. Recruitment of small NWP2 fish with a mean length of 26.8 ± 2.1 mm occurred during December to February, whereas NWP1 juveniles (30.9 ± 1.9 mm) were observed only in March. No modal progression in the length frequency distribution was observed in either species in Northern Taiwan (Fig. 5a).

In the south, recruitment of small NWP2 and NWP3 fish occurred in December–February. Slight modal progression was observed in the length frequency distributions, except for a modal shift that occurred between February and March (Fig. 5b). The NWP2 juveniles (37.1 ± 11.4 mm) were significantly larger than the NWP3 juveniles (27.2 ± 4.8 mm) from December to March (*t*-test, *P* < 0.01).

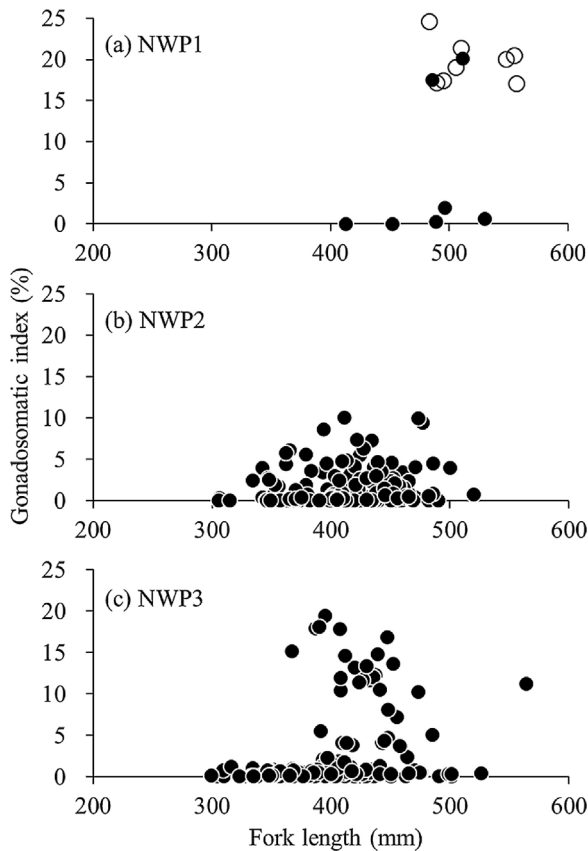


Fig. 3. Change in GSI according to size of (a) NWP1, (b) NWP2, and (c) NWP3 in Kaohsiung offshore area (open circle) and Kaoping River Estuary (solid circle). NWP: NW Pacific cluster.

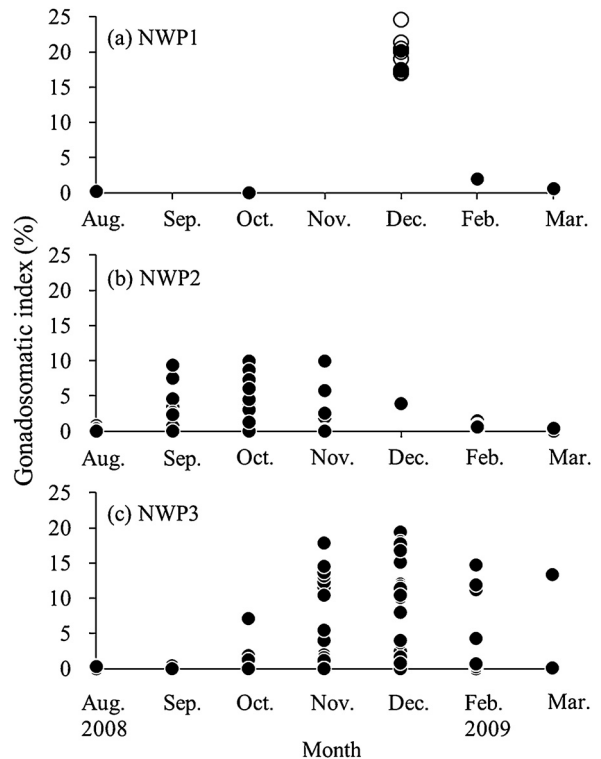


Fig. 4. Change in GSI according to month for (a) NWP1, (b) NWP2, and (c) NWP3 in Kaohsiung offshore area (open circle) and Kaoping River Estuary (solid circle). NWP: NW Pacific cluster.

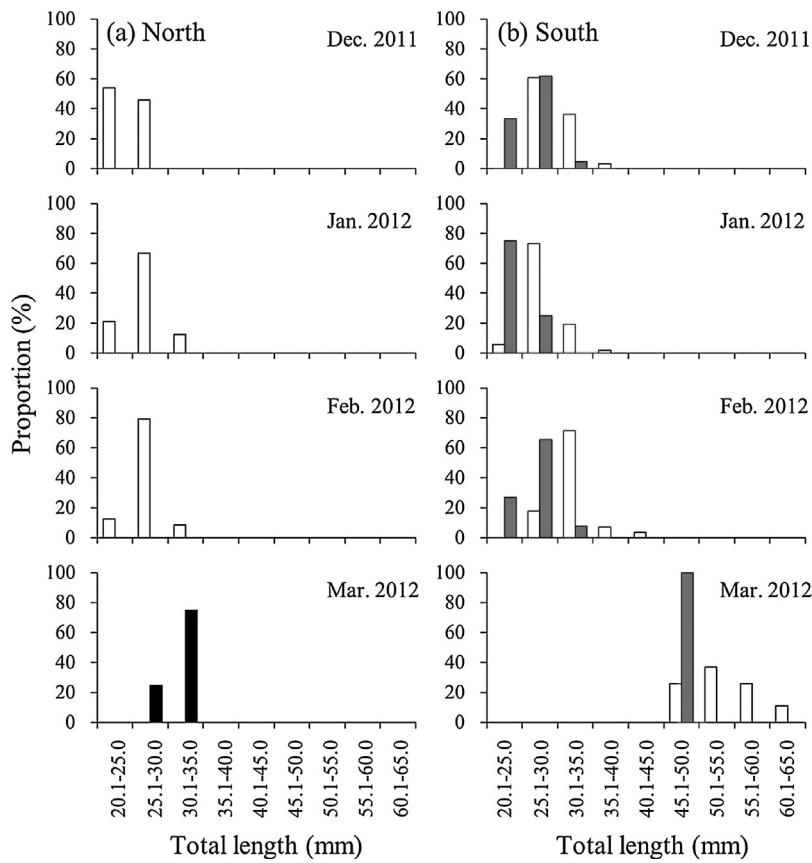


Fig. 5. Size distribution according to month for NWP1, NWP2, and NWP3 in (a) Gonshtian Creek Estuary, Northern Taiwan, and (b) Baoli Creek Estuary, Southern Taiwan. NWP: NW Pacific cluster. Black bar: NWP1; white bar: NWP2; and grey bar: NWP3.

4. Discussion

The contribution of reproductive barriers to the maintenance of the species boundaries among cryptic marine species is generally poorly understood. This study is the first to evidence that the genetic isolation of the three cryptic NW Pacific *M. cephalus* species that sympatrically occur in Taiwanese waters relates to differences in life history traits, delineating the degree of overlap of the species' spawning seasons and ecological niches.

4.1. Ecological niche segregation hypothesis

Several studies have investigated the life history traits of *M. cephalus* in Taiwanese waters; however, detailed information on the three cryptic species is lacking. Although previous studies did not recognize the cryptic species, they nonetheless suggested the coexistence, in Taiwanese waters, of fish in different stages of the life cycle that live in various aquatic environments [15,28–30]. According to recruitment and temporal patterns of fish landings, some authors suggested the existence of migratory and resident populations [23]. Some fish may remain in Taiwanese estuaries all year, whereas other fish may migrate offshore over a wide geographic area, from the south of Taiwan to the Japan and Yellow Seas.

According to the trace elemental composition of the otoliths of *M. cephalus* in Taiwanese estuaries, some *M. cephalus* were found to move between estuaries and offshore waters, whereas other fish moved between estuaries and freshwater habitats [28–30]. If the three migratory behaviors (one offshore and two coastal and estuarine migrations) correspond to species characteristics, then these behaviors indicate the existence of distinct ecological niches that limit hybridization. According to the species composition observed in the various aquatic environments sampled in our study and in previous studies [8,12], NWP1 is clearly the dominant species in marine environments, whereas NWP2 (as Species 1 [12]) and NWP3 are the dominant species in estuaries. No freshwater location has been sampled, thus limiting inference on the ecological niches of NWP2 and NWP3. Therefore, whether the freshwater migration inferred on the basis of otoliths corresponds to a specific ecological niche (for NWP3) or species plasticity (of NWP2 and NWP3) remains undetermined. Adults can migrate to freshwater environments to avoid competition, which suggests the existence of specific ecological niches in mugilids [30]. However, on the basis of the level of species admixture in some key habitats such as nursery and feeding grounds, whether the existence of a specific ecological niche is a prezygotic barrier sufficiently powerful to prevent hybridization is uncertain.

4.2. Reproductive isolation hypothesis

Our previous study [8] and the present study confirmed the large sympatry of the three cryptic species belonging to the *M. cephalus* species complex in Taiwanese waters, even though NWP1 appears to be more prominent in marine environments than are NWP2 and NWP3, which are dominant in estuarine environments. An absence of spatial isolation or incomplete ecological niche segregation does not necessarily imply that hybridization is possible. Species isolation may be only temporary, and species isolation that occurs during the reproductive period at locations such as spawning grounds represents a strong prezygotic barrier. Philopatry in fishes occurs in numerous species, including marine species [31–33]. During the reproductive season, 90% of the Atlantic herring *Clupea harengus* present in Newfoundland waters return to one of the three major spawning grounds in the Gulf of Maine [34]. If 10% of herring adults are vagrants and maintain gene flow, then an absence of genetic misassignment between mitochondrial and nuclear genomes in NW Pacific *M. cephalus* species suggests an absence of hybridization [8]. The yearly return of mature NWP1 fish to the offshore spawning ground in the Taiwan Strait supports the existence of specific spawning. The absence of other species also attests to the strong natal philopatric behavior. The problem that remains unresolved is determining the location of the spawning grounds for NWP2 and NWP3. The composition of NWP2 adults in the estuaries decreased as the GSI increased, suggesting that mature adults leave the estuaries; conversely, the composition of NWP3 adults remained stable in the estuaries, and their GSIs increased to values similar to those of NWP1 at their spawning grounds. This indicated that the NWP3 spawning grounds might be in or near estuaries, a phenomenon that is inconsistent with the typical life cycle of *M. cephalus* worldwide, which necessitates migration between estuarine and marine environments (for a review, see [14]). This phenomenon can explain why the NWP3 juveniles in the estuaries were the shortest; the juveniles of other species may have hatched at sea and spent more time recruiting in estuaries.

4.3. Temporal isolation hypothesis

Asynchrony of spawning periods is generally considered to explain the genetic isolation of species with overlapping distributions [35]. Such asynchrony has been observed in various marine organisms, including hermit crabs [36], bivalves [37,38], sponges [39], polychaetes [40], gastropods [41], and fishes [42–44]. Knowlton [35] listed 26 examples of spawning asynchrony in cryptic and sibling marine species. Regarding *M. cephalus*, asynchrony can also be considered a cause of species isolation because spawning periods are inconsistent between NWP1 and NWP2 and only partially overlapped between NWP2 and NWP3. Although the spawning period of temperate NWP1 overlapped with that of tropical NWP3, the reproductive behaviors of NWP1 differ completely. Therefore, the differences in the temporal evolution of the GSI and the juvenile recruitment in estuaries between species pairs

clearly suggest the importance of temporal isolation as a prezygotic barrier. Water temperature (21 °C) and short photoperiods (6 h of light and 18 h of dark) are likely crucial factors determining vitellogenesis in *M. cephalus* [45] and imply that spawning periods vary among the species of the *M. cephalus* complex located at different latitudes [14]. Thus, the spawning nonsynchrony of NW Pacific cryptic species is expected because these species are partially isolated over different latitudes. Similarly, some specific reproductive traits may result from the environmental conditions in which these species live outside the contact zone. Thus, the large-scale reproductive migration of the NWP1 species is likely directly caused by the large temperature decrease in the Japan and Yellow Seas during the northeast monsoon season. The onset of the northeast monsoon in winter leads to the emergence of the CCC, which blocks the northward incursion of the warm Kuroshio branch water, causing the temperature to decrease to 16 °C [46]. This temperature decrease drives the NWP1 species to migrate southward to find sea-surface temperatures conducive to spawning; in December, these conditions are present in the Taiwan Strait, where the respective warm and cold waters of the Kuroshio branch and the CCC mix [47].

Differences in the duration of spawning activity among species (the NWP1 breeding season is the shortest) can also be attributed to water temperature because high-latitude species have shorter spawning seasons than do their tropical relatives [48]. A previous study [8] emphasized that NW Pacific *M. cephalus* species have distribution ranges that overlap only partially; NWP1 prefer more temperate environments that do other species, whereas NWP3 are present mostly in tropical areas and NWP2 range throughout the warm Kuroshio Current in the NW Pacific. Thus, the extended spawning and recruitment seasons for the NWP2 and NWP3 species could be attributed to the minor seasonality of the environments in which their juveniles recruit.

4.4. Implications for fisheries and aquaculture management in Taiwan

In contrast to the notion that the *M. cephalus* historically fished on the spawning ground located in the Taiwan Strait belong to only one species, a previous study [12] and our studies have indicated that juveniles harvested in estuaries consist of a mixture of three species. A management policy that does not consider the presence of multiple species in a stock may have severe consequences on the species sustainability. The demographic productivity of the species may differ such that each species may not support equal levels of exploitation [34]. To ensure that these species are not overfished, improving the knowledge of the demographic characteristics of these species by monitoring each species individually is crucial [49].

In addition, because aquaculture and artificial breeding programs seem to be successful in slowing the decline in populations of *M. cephalus* worldwide [50–52], more comprehensively characterizing the physiological traits of these species is vital for improving aquacultural practices. The slight differences in ecological niches

observed in our study indicate that the existence of specific physiological requirements must be considered in aquacultural practice. For example, NWP1 may adapt more easily to cold water than do the other two species but adapt less easily to aquaculture ponds in Southern Taiwan where water temperatures are high. Differences in thermal tolerance among the Taiwanese species have been suggested. The migratory type (assumed here to be NWP1) of fish has an allele at the glucose-6-phosphate isomerase locus that differs from the most frequent allele observed in the resident type (probably NWP2) of fish, and the enzymatic activity of fish with these alleles varied under different thermal regimes [21]. Similarly, the freshwater affinity of the NWP3 species may substantially affect the ability of these species to grow in brackish aquaculture ponds. By contrast, NWP2 could not mature fully in estuaries because NWP2 fish with high GSIs were observed in the ocean [8]. Finally, understanding the physiological requirements of these cryptic species may improve aquacultural practices by reducing juvenile mortality.

5. Conclusion

Reproductive season and spatial segregation asynchrony between species in spawning grounds cause species isolation in the *M. cephalus* species complex. The importance of these two factors differs in the species delineation because the reproductive asynchrony is only partial. Philopatry and species segregation on spawning grounds are major prezygotic barriers in the *M. cephalus* species complex. Further investigation is warranted, particularly to determine the existence of specific ecological niches. The composition of these species varies among different coastal ecosystems; thus, more sampling is necessary for determining the fine-scale spatial and temporal distribution of these species, especially during the recruitment period.

Disclosure of interest

The authors declare that they have no competing interest.

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