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Research Paper

Life history and abundance of the minute triplefin, *Enneapterygius minutus* (Blennioidei, Tripterygiidae), from a seagrass tide pool on Green Island, Taiwan.

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Abstract

This study focuses on a small (maximum total length ca. 31 mm) and inconspicuous triplefin blenny species, *Enneapterygius minutus*, living in a specific tide pool with thick seagrass patches on Gui Wan Bi, Green Island, Taiwan. Its high abundance makes it the dominant species and a highly suitable candidate to monitor the condition of the seagrass ecosystem. Monthly collections were made during low tide from April 2007 to July 2008. Size-at-maturity and seasonal variation in reproductive effort were evaluated using GSI and histological analysis of gonadal tissue. A length-frequency analysis was obtained using the monthly data. *Enneapterygius minutus* is a short-lived species characterized by a maximum age of 351 days. It has low batch fecundity (average = 140 eggs) and may breed year round with a high peak during the spring. Recruitment of juveniles began about one to two months after breeding occurred. Data also show that *E. minutus* uses seagrass meadows as habitat for hatching and growth. It is suggested that *E. minutus* may be used as a species to reflect the community condition of the seagrass meadow on Green Island under the increasing thermal stress.

Key words: indicator species, otolith microstructure, reproductive biology, seagrass ecosystem

Introduction

Seagrass meadows cover about 0.1 − 0.2% of the global ocean, and belong to an important type of coastal ecosystem (Duarte 2002) that plays indispensable roles in the environment including producing and exporting organic carbon, nutrient recycling, stabilizing sediment, and enhancing biodiversity, such as providing nursery ground for the juvenile and sub-adult fishes (Orth et al. 2006, Pollard 1984). Climate change, especially global warming, can exert stress on this ecosystem by increasing water temperature, rising sea level, and reducing productivity due to salinity change, etc. (Short and Neckles 1999). Due to its ecological importance, this ecosystem deserves attention now as global warming has become a threat.

In Taiwan and her nearby offshore islets, seagrass meadows are found in Kenting Peninsula (in southern Taiwan), Liuchiu Yu (an islet off the southwestern coast of Taiwan), Green Island (off the eastern coast of Taiwan), and the Pescadores (or Penghu) in Taiwan Strait. Apart from the Pescadores, all are close to where the Kuroshio Current branches near Taiwan. The seagrass meadows in the Kenting Peninsula and Liuchiu Yu are in tidal flats and very few fishes could be found during low-tide period. However, seagrass meadows on Green Island are spread out not only in tidal flats

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but also in some tide pools and are in better growing condition. Only three sites in Green Island have been found with seagrass meadows. During low tide, some fishes remain in the tide pools with seagrass patches, including the minute triplefin (*Enneaptergius minutus*), a small (with a maximum total length of 30.9 mm) and cryptic triplefin tripterygiid species, which is very abundant in the seagrass tide pools at Gui Wan Bi on Green Island. However, it is absent from the seagrass meadows at other two parts of this island.

The minute triplefin is distributed in the Indo-West Pacific and ranges from American Samoa, north to Ryukyu Islands, and south to Australia (Fricke 1997). These small fish are inhabitants of tide pools, large inshore pools, lagoon side of reefs, dead coral reef flats, limestone rocky shores at low tide, sandy beaches with some beach rock, and tidal channels. They live at depths ranging from 0~5 meters. No report of its presence in seagrass meadow appears in the literature before (Fricke 1997).

Indicator species are often chosen for monitoring because they represent a particular ecosystem or management concern (Soule and Kohm 1989) or are easily sampled, sorted and identified. They should bear some of the following attributes: relatively high abundance (i.e., always present and easy to locate in the field); well-known taxonomy and easy identification; good background information (e.g., on life history, genetics, behavior, ecology, biogeography); functional importance within the ecosystem; predictable, rapid, sensitive, analyzable, and linear response to disturbance; close association with and identification of the conditions and responses of other species (Stork and Samways 1995). Because of the persistent presence and abundance of minute triplefins in seagrass meadows on Gui Wan Bi, this fish may be a suitable indicator species to be monitored to assess the seagrass-ecosystem at this place. Change in distributions, abundances, and demographic characteristics (e.g., population, sex, and age structure) of this indicator species may signal impending adverse changes in the ecosystem as a whole (Stork and Samways 1995). Due to a lack of information, the minute triplefin still does not fulfill all the above-mentioned attributes of indicator species (e.g., good life history information, and an understanding of the species functional importance within the ecosystem, responses to environmental disturbance, and association with other species within the seagrass meadow). Therefore, further study on this species is required. It is, therefore, the aim of this study to generate some of this information.

To evaluate the ecological processes (e.g., energy flow in the food web or species composition) in a seagrass ecosystem, information on population demography and autecology of the dominant species are required (Longenecker and Langston 2005). The life histories of small, cryptic fishes, which are often very abundant and specious in coral reefs, are largely unknown (Longenecker and Langston 2005). Similarly, few works have been published about tripterygiid species: Longenecker and Langston (2005) studied the life history of *Enneapterygius atriceps*; De Jonge and Videler (1989) studied the reproductive biology and mating strategy of *Tripterygion triperonotus* and *T. delaisi;* Thompson (1986) studied the spawning success and mate choice in *Forsterygion varium.* Length-weight relationships for seventeen tripterygiid species (not including the minute triplefin) have been estimated and are available from the literature, both published and on-line (e. g. www.fishbase.org).

The aim of this study was to describe the reproductive, age and growth patterns, and life history of the minute triplefin, which is the dominant species in some seagrass meadows on Green Island.

Materials and methods

Field collection

All specimens were collected once a month from a specific tide pool contains with seagrass (*Thalassia hemprichii*) at Gui Wan Bi (22°20'N 120°23'E), Green Island (Fig. 1) from April 2007 to July 2008. This tide pool is about 21m^2 in total area and the depth of the water during low tide ranged between 10 and 60 cm. This tide pool is connected to other parts of the tidal flat after low tide that triplefins from the adjacent seagrass patches or tidal flat can move in. A fixed amount of rotenone (about 150 g in 10 L of water) was applied to the tide pool and two persons collected the specimens using dip nets. Consecutive monthly samples were at least two weeks apart to allow for recruiting of triplefins. All collections were made during low tide in the daytime. Specimens were fixed in 10% formalin immediately upon capture. Standard length, body weight, and sex for each individual were measured. Body length and weight were measured to the nearest 0.1 mm and 0.1 g, respectively. Fecundity, egg size, and numbers of mature individuals were assessed.

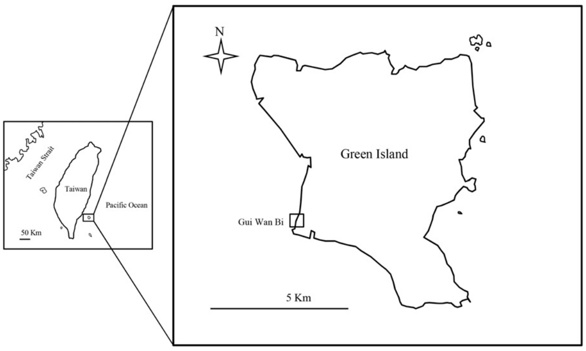

Fig. 1. Location of the sampling tide pool at Gui Wan Bi, Green Island, Taiwan.

Age and growth

Sagittae were used to describe the age and growth (Longenecker and Langston 2005). After sagittae were removed from the skull, they were put into a flat embedding mold and left to air dry. Then, the mold was filled with thermoplastic glue and left to harden at room temperature for about a day. After sagittae were fixed, they were ground on a series of 1200 and 2400 grit sandpaper until the core of the sagitta could be seen clearly under the microscope; they were then polished with alumina slurry on the felt.

Otolith increments were counted on the computer after the sagittae photographed at 200 × with a camera attached to a microscope. Mean standard length and mean age for specimens from apparent 10-day age groups were calculated. The relationship between otolith size and the average standard body length of 10-day age fish groups was determined by the radial length of the sagitta measured from the core to the anterior margin along the anterior-posterior axis. The distance from the core to different increments was also measured for length-at-age back calculation outlined in Carlander (1982). A von Bertalanffy growth curve was constructed using the method outlined in King (1995): L_∞ was estimated using a Ford-Walford plot of standard length data, whereas a von Bertallanffy plot of ln (L_∞- Lt) vs. t was used to compute K and t_0. The relationship between standard length of the fish and radial length of the sagitta was determined by linear regression analysis. A settlement mark was indicated by a color change in the saggitta (Longenecker and Langston 2005, Wilson and McCormick 1999.). The relationship between total body weight (W) and standard length (SL) was examined by the formula $W = q(SL)^b$, where q and b are constants (King 1995).

Reproduction

The gonad weight and total body weight of each female specimen examined were used to calculate gonadosomatic index (*GSI = gonad weight/total body weight* × 100) to describe seasonal variation in females' fecundity (i. e., its reproductive investment). The total number of eggs larger than 0.1 mm in each female specimen was counted under a dissecting microscope for fecundity analysis.

Size at sexual maturity of females was determined via histological examination of gonads. Because developmental stages of testes were difficult to determine, a dark-body-color-pattern was adopted as a diagnostic character for mature male (Fricke 1997). Males and females of all species in the genus *Enneapterygus* have quite different body colors, and it is easy to identify them in natural condition or even after preservation. Moreover, the gonads of mature males matched with the dark-body-color-pattern.

Gonads removed from female specimens were dehydrated through a series of graded ethanol baths and embedded in paraffin wax after two changes of xylene. A cross-section of gonads (9μm) were mounted on slides, stained with hematoxylin and eosin, and covered with cover slips and permount.

Oocyte development was classified into seven stages (Wallace and Selman 1981): chromatin nucleolar, perinucleolar, yolk vesicle, early yolk, late yolk, migratory nucleolus and hydrated oocyte. The presence of postovulatory follicle was also noted.

Ovaries were classified according to the most advanced oocyte stage present: Phase I − resting: the oocytes in the ovary are all at chromatin nucleolar and perinucleolar stages. Phase II − early development: most of the oocytes were in yolk vesicle stage. Phase III − late development: the most advanced oocytes were in vitellogenesis. Phase IV − maturation: migratory nucleolus and hydrated oocytes can be seen in the ovary. Phase V − spent: postovulatory follicles were present in the gonad. Females with eggs in vitellogenesis or later phases were considered mature.

Oocyte diameter at late vitellogenesis was measured under a dissecting microscope to the nearest 0.01 mm and these data were grouped into 0.05 mm size classes. All oocytes of a female greater than 0.1 mm in diameter from a single ovary were counted. A histogram of these data revealed a size distribution in oocyte diameter to find out the relationship between oocyte diameter and oocyte stage.

The methods of King (1995) were followed to construct a logistic curve to estimate size at maturity (i.e. the length at which 50 per cent of all individuals of a given sex are mature is called the mean length at sexual maturity, L_m).

Results

An assemblage of fish species at the sampled seagrass tidepool

Besides the 862 tripterygiids, fishes from 5 other fish families were collected from the tidal pool. These miscellanea include labrids, gobiids, atherinids, pomacentrids, and muraenids were less than 10% of total fish specimens. In other words, *E. minutus* was the most abundant species found in the seagrass tide pool.

Age and growth

The otolith radial lengths (ORL) of 39 individuals were plotted against the standard length (SL) of the fish. Formula of the linear regression was $SL = 0.0282(ORL) + 4.1701$ (R^2 =

0.778; P<0.001). The relationship between total body weight (W) and standard length (SL) can be described by a power function, $W = 0.0071(SL)^{3.282}$.

Each otolith ring in *E. minutus* was assumed to represent 1 day of age (Jones 1993, Longenecker and Langston 2005). The settlement mark indicated by an abrupt change in color from the otolith microstructure suggests that most settlements occur at the 43rd day (Fig. 2). The parameters of von Bertalanffy growth curve obtained from the Walford plot were $L_\infty = 30.9mm$, $K = 0.0054d^{-1}$, and $t_0=-24.75d$, thus the von Bertalanffy growth formula for this species in the seagrass bed at Green Island is $L_1= 30.9[1\text{-exp}^{-0.0054(t+24.75)}]$(Fig. 3). The largest specimen collected was 26.8 mm in standard length. On the basis of the von Bertalanffy growth formula, the oldest fish in our samples would have lived for 351 days.

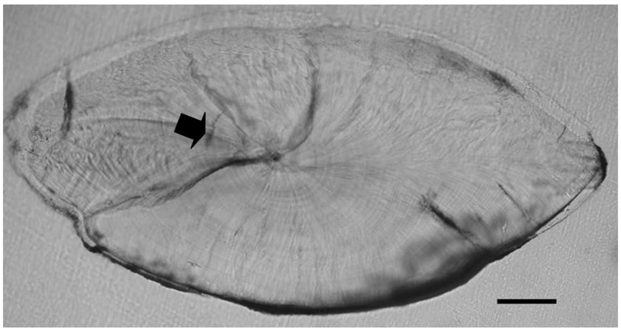

Fig. 2. A transmitted light micrograph of a ground and polished sagitta from an *E. minutus* (SL = 18.25 mm). Arrow points to a color change pattern presumed to be a settlement mark. Scale bar = 100μm.

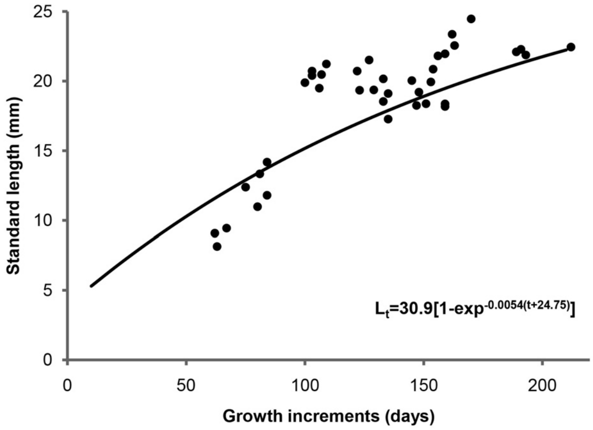

Fig. 3. The von Bertalanffy growth curve of *E. minutus*; $L_\infty = 30.9$ mm, $K = 0.0054$ d^{-1}, and $t_0 = -24.75$; n = 39.

Reproduction

Enneapterygus minutus with dark body color were assumed to be reproductively mature males (Fricke 1997). Assuming this is correct, fifty percent of males were sexually mature by 18. 0 mm (N = 294). Mature male could be identified as small as 15.5 mm. Mature females were as small as 16.3 mm. Fifty percent of females were mature at 19.4 mm (N = 250). When combining both sexes, fifty percent of all individuals were mature at 18.7 mm (N = 513). The sex ration of males: females = 1.57: 1.

The batch fecundity of *E. minutus* is quite small. Their maximum egg number was around two hundred (normally ranging from 100 to 150, but some had fewer than 100 eggs) (Fig. 4). A cubic function, $BF = 0.0035(SL)^{3.4467}$, can be used to describe the relationship between batch fecundity and standard length (Fig. 5).

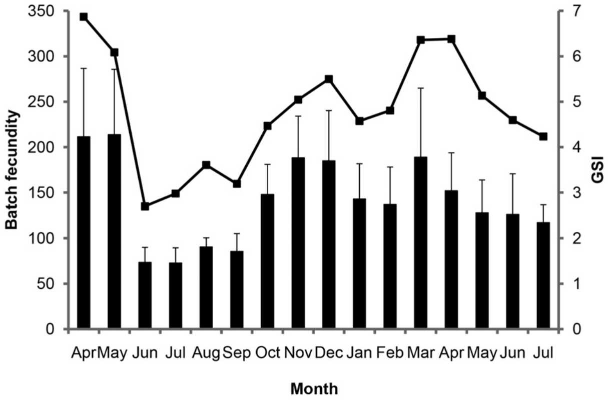

Fig. 4. Seasonal trend in gonadosomatic index (GSI) (line) and batch fecundity (bar) for female *E. minutus* (N = 115).

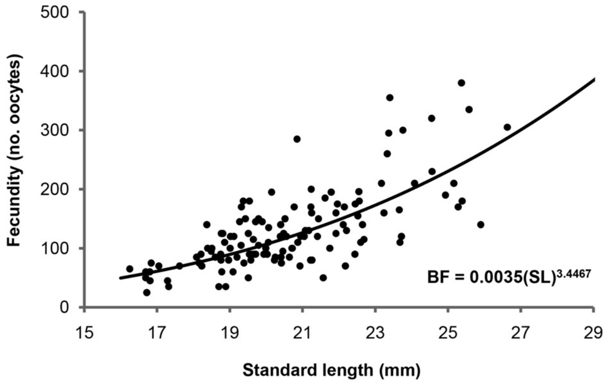

Fig. 5. Relationship between standard length (SL) in mm and batch fecundity (BF) for female *E. minutus*;
$R^2 = 0.520$; n = 127.

Ovaries of mature females contained all stages of oocytes. Development of oocyte is classified into seven stages (Fig. 6). Histological examination of the developmental oocyte stages suggested that oocyte larger than 0.4 mm in diameter may represent the spawning batch (Fig. 7). These oocytes were assigned to phases III and IV, whereas those smaller than 0.4 mm were assigned to phases I and II. The oocytes at phases III and IV were mostly gathered in the center of the ovary while the oocytes from phases I and II surrounded the edge. Typically, ovaries of mature females could fill the whole visceral cavity, and one can even see the oocytes under the microscope through the abdomen.

According to the monthly frequency distribution of the ovarian developmental stages (Fig. 8), together with seasonal trend in gonadosomatic index (GSI) and batch fecundity of female gonads (Fig. 4), breeding in *E. minutus* extended throughout the year. But, there is a quite obvious seasonal surge in breeding activity during the spring. There is a positive correlation between the GSI value and batch fecundity (r = 0.57; n = 115).

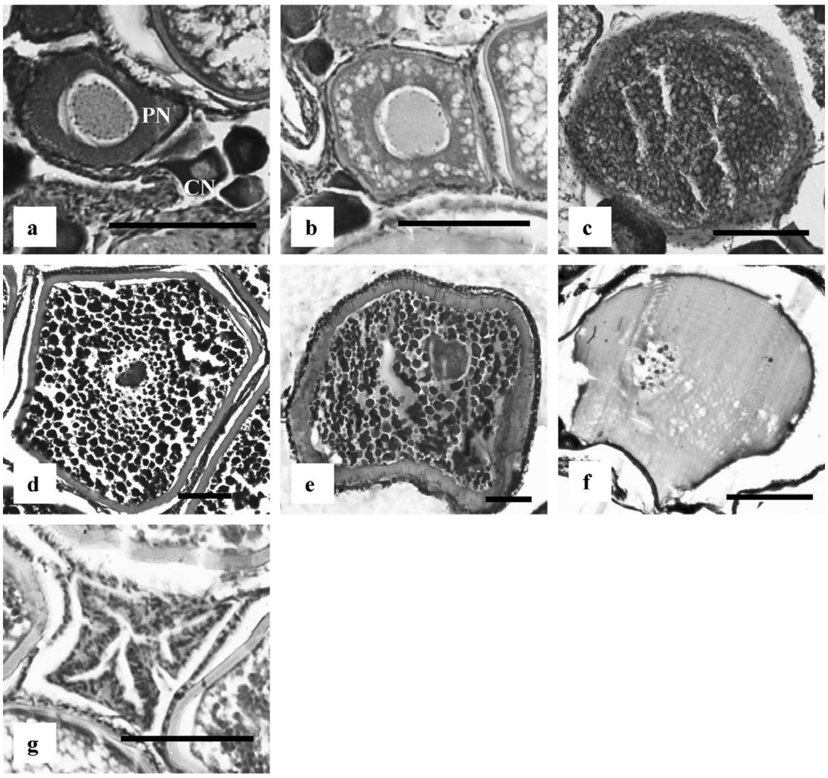

Fig. 6. Histological sections of different oocyte development stages of *E. minutus*: (A) Chromatin nucleolar (CN), Perinucleolar (PN); (B) Yolk vesicle; (C) Early vitellogenesis; (D) Late vitellogenesis; (E) Migratory nucleolus; (F) Hydrated oocyte; (G) Postovulatory follicle. Scale bar = 100 um.

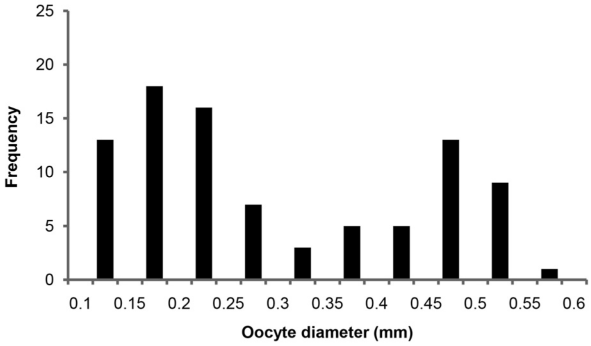

Fig. 7. A size-frequency histogram of oocyte diameters from a gravid 22-mm *E. minutus* (152 oocytes were examined).

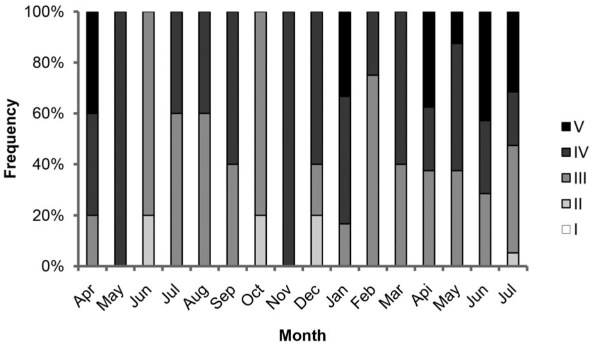

Fig. 8. Monthly frequency distribution of developmental stages of female gonad in *E. minutus*; n = 105.

Recruitment of juvenile

Juveniles (immature individuals) comprised the lowest percentage of total population size from August to October, but their relative abundance reached 40% or more in other months (Fig. 9). There is a quite regular length frequency distribution pattern in samplings (Fig. 10). Although juvenile individuals could be found throughout the year, the number was higher in the months after peak breeding, with July, January and May, showing an increase in the relative abundance of juveniles (Figs. 4, 9, 10). There were obvious spawning groups found from the histogram: they started from May 2007, October 2007, February 2008 and May 2008, respectively (Fig. 10).

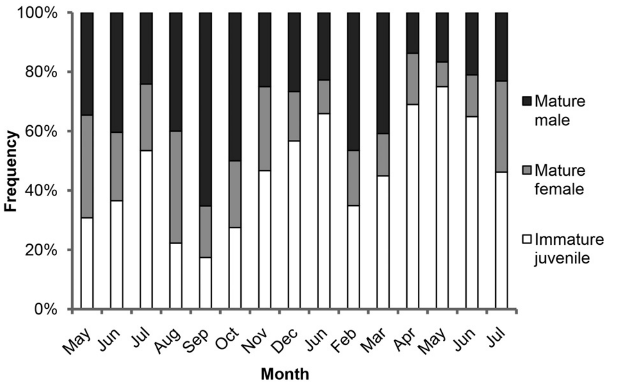

Fig. 9. Monthly distribution frequencies of the population composition in *E. minutus;* n = 82.

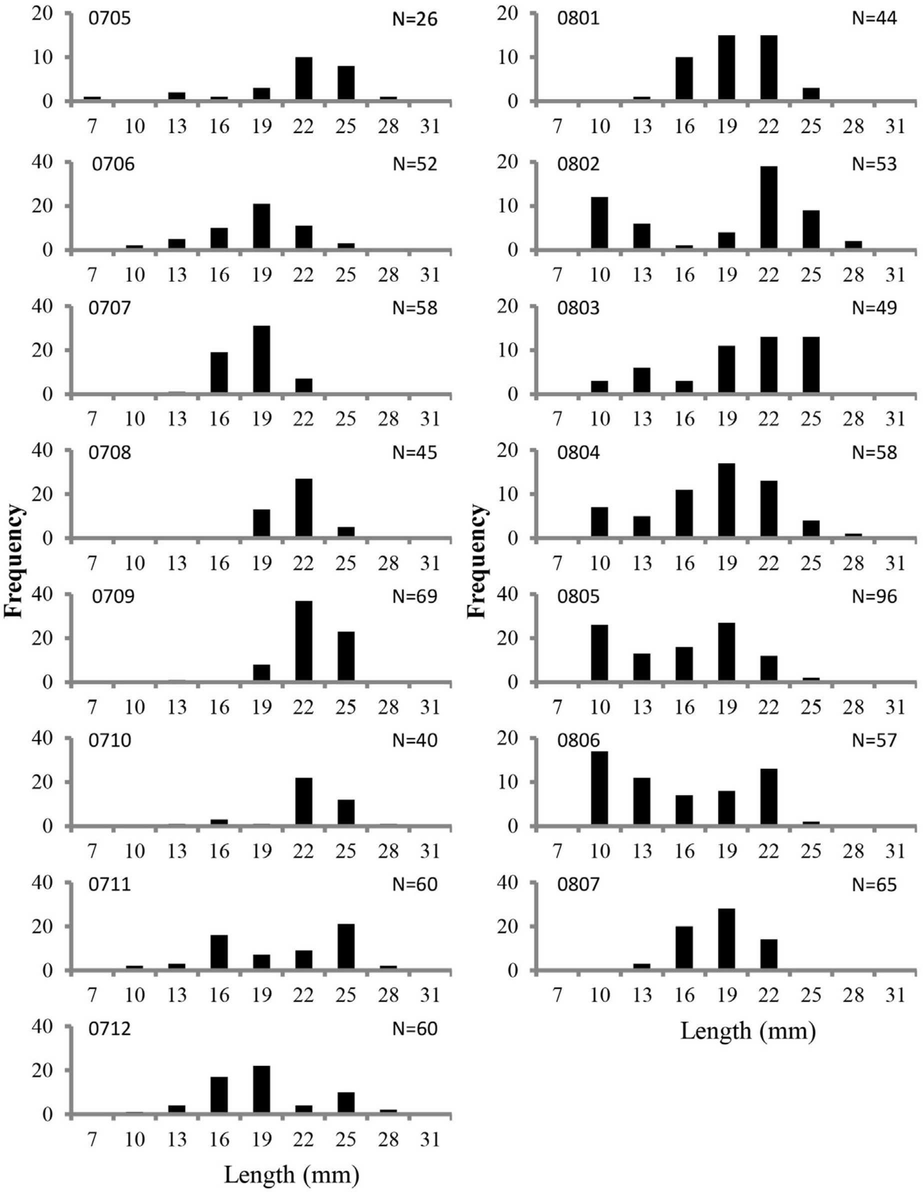

Fig. 10. Length-frequency histograms of *E. minutus* from May 2007 to July 2008.

Discussion

In Taiwan and her nearby islands, the minute triplefin has been recorded in Liu-Chiu Yu and Green Island. However, from our investigations throughout the seagrass meadows around Taiwan, the minute triplefin has only been found in seagrass tide pools at Green Island. More importantly, its number in this habitat in Green Island is more abundant than those in other tidepools without seagrass. Because of this difference, minute triplefin is inferred to prefer seagrass meadows over other niche types.

As in previous work on triplefins, otolith ring formation is daily (Longenecker and Langston 2005). On this basis, the settlement of minute triplefin larvae takes place ~ 43 d after hatching. The minimum mature age of the minute triplefin (18. 7 mm) at the seagrass meadows on Green Island could be estimated using the von Bertalanffy growth formula as ~ 147 d. The oldest individual estimated from the collected individuals was 351 days. These estimates, combing with those of *E. atriceps* in Hawaii (settlement at ~ 30 d, mature age at ~ 55d; oldest individual lived for ~ 190 days), shows that the triplefins are relatively short-lived species.

Although the weight and size of the gonad increase with growth, the development of the gonad may not perfectly match body size. If only the peak of the GSI value was used as a standard for estimating maturation, it may not actually reflect the mature stage of the gonad. Therefore, it is much more reliable to use the GSI value together with the histological characteristics of the ovary to determine the maturation of the gonad.

The low batch fecundity in minute triplefin, however, is an interesting phenomenon. A study by Thompson (1986) has shown that the clutch size of larger triplefin in temperate areas can reach 1500. It is unlikely that an *E. minutus* female would release all the eggs in her ovary in a single spawning event; it is considered to be more adaptive for the female to mate with multiple males in a whole stretch of time. Moreover, the large egg size and low number of oocytes suggest that the minute triplefin might have parental behaviors that increase the survival rate of hatchings. These strategies, especially male guarding behavior, had been reported by Mayr and Berger (1992) and Thompson (1986) in some intertidal fish and the mottled triplefin.

The decreasing of GSI value and batch fecundity during May 2007 and April 2008 is associated with the increase in juvenile abundance occurring about one month later. For this reason, the temporal appearance of the minute triplefin juveniles matched with that of the GSI values with a lag of one to two months. This evidence seems to match the otolith analysis that the recruitment occurs one and half months after spawning.

Because of the close relationship between the minute triplefin and seagrass meadows on Green Island, this species is a good candidate to reflect environmental stresses such as climate change and global warming. Any damages to the seagrass meadows could also lead to changes in the residential fish assemblage and drops in abundance as they play an important role in fish nurseries where small fishes can find shelter from predators and provide abundant food sources (Pollard 1984). Due to the short life span and low batch fecundity of *E. minutus*, its population size will likely respond rapidly to improving or declining environmental quality. This characteristic renders it as a suitable indicator species. The present dataset on the minute triplefin on Green Island can serve as the baseline data for future comparisons.

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