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Reproductive Biology of Invasive Knifefish (*Chitala ornata*) in Laguna de Bay, Philippines and its Implication for Control and Management

Jhon Michael C. Castro[1, *], Ma. Vivian C. Camacho[1, 2], and Jon Carlo B. Gonzales[2]
[1]*Animal Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines-Los Baños, College, Laguna 4031, Philippines*
[2]*Limnological Station, University of the Philippines-Los Baños, College, Laguna 4031, Philippines*

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ABSTRACT

A full understanding of life history characteristics of invasive species is a fundamental prerequisite for the development of management strategies. *Chitala ornata* (knifefish) have established highly abundant and destructive populations in Laguna de Bay (Philippines). In the present study, we examined the reproductive biology of *C. ornata* with the aim of improving the efficiency of management strategy. Gonado-somatic indexes and gonadal analysis showed that knifefish spawned from February and August. They spawned fewer and larger eggs than native fish species in the lake and provides parental care. The adult sex ratio was male skewed while sexual size dimorphism was female skewed. The different reproductive traits appear as a crucial biologic aspect for developing control programs. Specifically, control measures should be implemented and/or intensified prior to spawning season of *C. ornata* from December to March. Removal strategies should also consider habitat segregation of *C. ornata* sexes during spawning season. The body size of the smaller males is the determinant for minimal mesh size of the nets used in physical removals of *C. ornata*.

Key words: *Chitala ornata*, Reproductive Biology, Invasive Species Management

INTRODUCTION

Freshwater ecosystems touch nearly all aspects of human society, acting as centers of organization within the landscape, providing countless cultural and ecological services, and supporting a rich diversity of biological life. The escalating need to simultaneously meet the water demands of a growing human population and ensure ecological integrity is largely the reasons why freshwater organisms are among the most imperilled fauna worldwide (Jenkins, 2003). Laguna de Bay is the largest freshwater body in the Philippines and harbours a total of 33 fish species consisting of 14 indigenous, 5 of which are migratory, and 19 exotic or introduced (Llasco & Espaldon, 2005). Of the 9 non-migratory indigenous species only 5 are presently caught in significant quantities (Palma *et al*., 2002) namely: *Arius manilensis*, *Leiopotherapon plumbeus*, *Glossogobius giuris*, *Channa striata*, and *Gobiopterus lacustris*. However, population of these indigenous fish fauna of Laguna de Bay is currently under threat due to the introduction and subsequent establishment of invasive fish species (Cagauan, 2007), which includes *Chitala ornata* or clown knifefish.

Chitala ornata (Gray, 1831) is a carnivorous fish that is indigenous to Mekong River (Poulsen *et al*., 2000). It was introduced in the Philippines primarily for ornamental purposes only (Cagauan, 2007), however, several individuals have found their way into the lake either by escaping form ornamental fish farms or aquarium or deliberately released by some fish owners and later successfully established their population in the lake. The clown knifefish is considered as a nuisance species in Laguna de Bay because of its voracious feeding habit which includes prey that are of economic importance (e.g. *Chanos chanos*, *Oreochromis niloticus*, *Cyprinus carpio*).

Non-native invasive fish species are increasingly recognized as a significant contributor to extinction threat in fresh waters, one that joins and combines synergistically with habitat loss and fragmentation, hydrologic alteration, climate change, overexploitation, and pollution (Dudgeon *et al*., 2006). Although not all introduced fishes become established, and the fraction of those that do often have little appreciable effects on their new ecosystems, many others exert significant ecological, evolutionary, and economic impacts (Ricciardi & Kipp 2008). Primary suppression methods used for non-native fish suppression in lakes include netting, chemicals, migration barriers, and electricity. Methodologies such as gill netting, piscicide application, or movement barriers are costly and have significant negative environmental effects (Martinez *et al*., 2009). Unintended consequences include catch of non-target organisms with gillnetting, mortality of non-target organisms from the use of piscicides, food-web alterations, and the obstruction of native fish spawning migrations and nutrient distribution in a watershed.

*Corresponding Author's E-mail: jccastro5@up.edu.ph

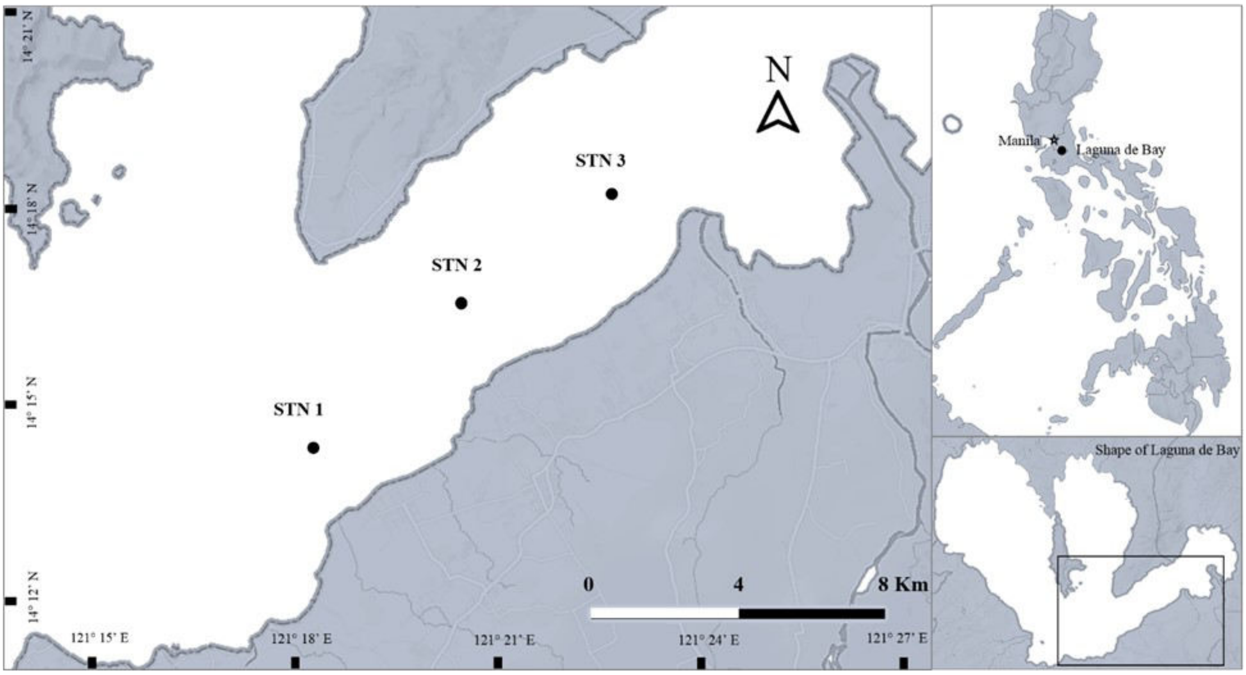

Figure 1. Sampling sites (*filled circle*, STN1 – STN3) for *C. ornata* in the West bay of Laguna de Bay.

Another approach in attaining control over invasive species is by identifying those aspects of its life history including its reproductive biology, which contribute most to its population growth rate (Loppnow *et al*., 2013). Therefore, quantifying the reproductive biology of *C. ornata* outside of their native range may promote the development of a more effective management strategy. Here, the reproductive biology of the *C. ornata* was assessed specifically its seasonal spawning cycles, trade-offs between egg size and fecundity, adult sex ratio and sexual size dimorphisms (SSD).

MATERIALS AND METHODS

Study Area

The present study was conducted in Laguna de Bay, also known as Laguna Lake (121°3-28' E, 14°10-31' N). It occupies an area approximately 900 km^2 with a shoreline of 220 km. It has an average depth of 2.5 m and a maximum water holding capacity of 2.9 billion m^3. Geographically, the lake can be divided into West bay, East bay, South bay, and Central bay.

Fish Sampling and Data Collection

From May 2015 – April 2016, *C. ornata* samples were collected monthly using drag seine net with stretched mesh size of 20 cm. in three sites located in East bay of Laguna de Bay (Figure 1). Four nets were deployed per site and per month from 4:00 to 6:00 a.m. The nets were retrieved by slowly pulling the attached rope. All knifefish caught in the net were collected. All *C. ornata* collected from three sampling sites were pooled in each month. Samples were processed within 24 hours after they were caught. Total body length and body weight were measured for each individual to the nearest 0.1 cm and 0.01kg. Based on macroscopic appearances, gonads of both sexes were classified into five reproductive stages (stage I-V) following Brown-Peterson *et al.* (2011). Developmental stages of ovaries and tests were assessed by visual inspection primarily based on its appearances (Table 1). Gonads (≥stage II, Table 1) were carefully removed and weighed to the nearest 0.01 mg. The sex (female or male) of each individual was determined by the macroscopic differences of ovaries and testes (≥stage II, Table 1). To estimate female fecundity, about 5.0 g of mature ovaries (stage IV at breeding season, Table 1) was sampled from the anterior, middle, and posterior sections of each lobe. The samples were weighed and fixed with 10% formalin solution for 2 weeks and then preserved in 75% ethanol. The number of eggs in each subsample was subsequently determined and the length and of the eggs measured under an optical microscope.

Statistical Analyses

Gonadosomatic Indexes (GSI) of both mature female were computed using this formula; GSI = (Gwt/Bwt) *100, where Gwt = gonad weight, Bwt = body weight. Absolute Fecundity was determined as the product of the gonad weight and oocyte density. Oocyte density is the number of oocytes per gram of ovarian subsample tissue. Relationship between absolute fecundity and total body length was assessed using Pearson r. Adult sex ratio was calculated and the significance of deviation from 1:1 null hypothesis was tested using Chi-square test ($P<0.05$). Mean lengths relative to total length of each metric variable between sexes were compared using Wilcoxon-Mann-Whitney test ($P<0.05$).

Table 1. Macroscopic characteristics (modified form Brown-Peterson *et al.* (2011) used to classify gonad maturity stages of *C. ornata* in Laguna de Bay.

Gonad maturity stage	Female	Male
I (virgin stage)	Sexes indistinguishable by naked eye; gonad is small, often clear, blood vessels indistinct	Sexes indistinguishable by naked eye; gonad is small, often clear and threadlike
II (immature stage)	Ovaries are small, translucent or pale yellow, oocytes are indiscernible	Testes are thin, white or gray and larger than stage I
III (maturing stage)	Ovaries are swollen, orange in color and occupy up to 1/3 of the body cavity; oocytes are tightly packed in ovaries, and can be visible from epithelium	Testes are firm and ivory-white
IV (spawning stage)	Ovaries occupy 1/3 to 2/3 of body cavity; oocytes attain maximum volume	Testes are large and ivory-white
V (spent stage)	Ovaries are flaccid and sometimes red	Testes are flaccid and decrease in volume

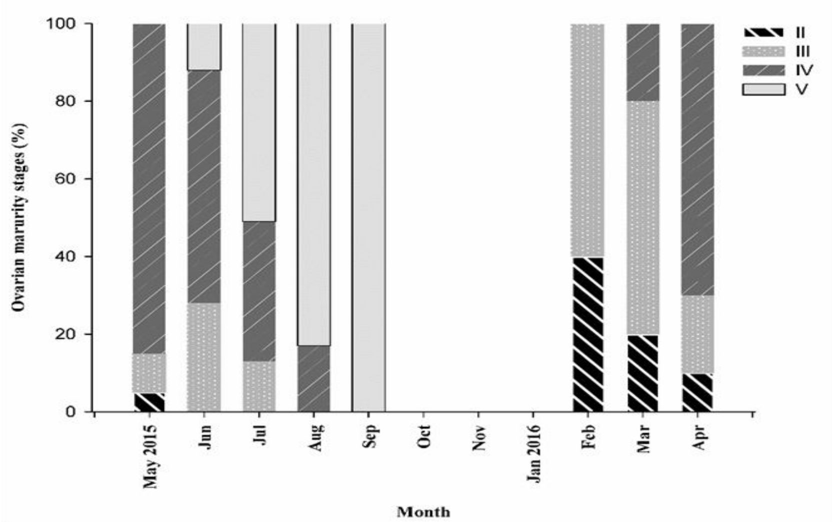

Figure 2. Monthly changes in the percentage of ovarian maturity stages for *C. ornata* from May 2015 – April 2016 in Laguna de Bay. Absence of data indicates that individuals' sex was unidentifiable.

RESULTS AND DISCUSSION

Spawning Season

Chitala ornata spawned from March to August with spawning peak occurring in April to May. Specifically, the maturity stage of most ovaries of knifefish were at stage I (virgin) during October to January. Individuals with stage IV ovaries (spawning) started to appear in March. The proportion of stage IV ovaries were more than 50% during the months of April, reaching its peak of 85% in May and decreased gradually from June to August (Figure 2). The gonado-somatic indices of female knifefish were high during February to August, with its peak recorded in March (2.87%). Mean gonado-somatic index of female knifefish during its spawning season was 1.76% (Figure 3). Spawning cycle of *C. ornata* in Laguna de Bay was longer compared to its native range of Mekong river where knifefish were reported to spawn on submerged wood from March to July (Poulsen *et al.*, 2004). Knowledge in the spawning seasonality of an invasive fish species is an invaluable instrument to optimize the timing the implementation of management strategies (Britton *et al.*, 2011). To prevent increase in population of by several orders of magnitude and extensive dispersion of non-native fish at every end of spawning season, management operations for fishes may often be most effective when implemented pre-spawning period (Wimbush *et al.*, 2009; Britton *et al.*, 2011). Moreover, the removal of adults soon after spawning may result in a population increase in juveniles (e.g., decreased intraspecific competition) rather than their decline. One of the striking example is in New York, the mass removal of 47,474 *Micropterus dolomieu* (smallmouth bass) in over a 6-year period from an Adirondack lake initially reduced *M. dolomieu* abundance by 90%, but ultimately resulted in increased abundance (Weidel *et al.*, 2007; Zipkin *et al.*, 2008). Decreased intraspecific competition led to accelerated maturation of juveniles and, ultimately, improved recruitment and population increase (Ridgway *et al.*, 2002; Zipkin *et al.*, 2008). This phenomenon is known as the hydra effect or overcompensation (Zipkin *et al.*, 2008; Abrams 2009; Strevens and Bonsall 2011).

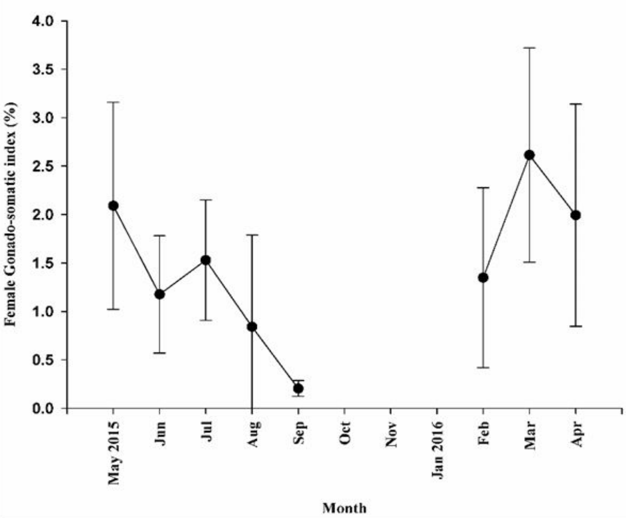

Figure 3. Monthly changes in the gonado-somatic indices of female *C. ornata* from May 2015 – April 2016 in Laguna de Bay. Absence of data indicates that individuals' sex was unidentifiable. Mean ± SD.

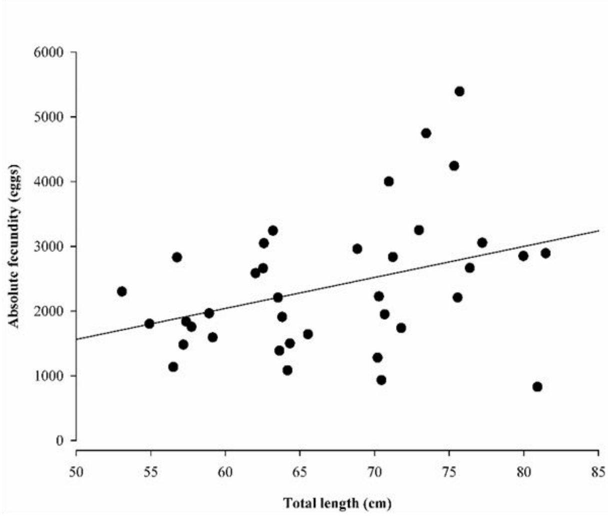

Figure 4. Relationship between total body length and absolute fecundity of female *C. ornata* (r = 0.36, n = 37) from May 2015 to April 2016 in Laguna de Bay.

Egg size and fecundity

Knifefish produce eggs averaging 4.02 mm in diameter (n = 256) with a mean absolute fecundity of 2379 (± 1031, n = 37). *C. ornata* eggs are larger than that of native fish species of Laguna de Bay such as *G. giuris* and *L. plumbeus* except to eggs of *Arius manillensis* with eggs averaging 9 – 12 mm in diameter. The absolute fecundity of female *C. ornata* showed weak positive relationship to total body length (Figure 4; r =0.36). Reproductive investment in fishes can be examined based on egg size, which is related to provisioning of the egg (Moodie *et al*., 1989) and ultimately impacts embryo (Johnston, 1997) and larval survival (Berkeley *et al*., 2004). Care-giving species like *C. ornata* tends to produce larger eggs than non-carers and their extent of care also appears to correlate positively with egg size (Sargent *et al*., 1987). Larger embryos require more care, primarily because they take longer to develop and require more oxygen (Shine, 1989). However, having large eggs and longer incubation time can be a disadvantage to fishes when employing electricity as a means of control of their population. It has been shown that vulnerability of embryos to electrofishing is positively related to its diameter (Dolan & Miranda, 2004). Research on the lethal effects of electrical fields in embryos of numerous fishes indicate that embryos are most vulnerable to DC electrical fields, vulnerability varies with stage of embryonic development; and even brief exposure is sufficient to induce high mortality in embryos during critical developmental stages (Henry *et al*., 2003).

Adult sex ratio

A total of 192 mature knifefish individuals were collected, of which 55 were female and 137 were male, resulting to an overall ratio of 1:2.49, respectively, which significantly deviated from expected ratio of 1:1 (χ^2=35.021, df=1, P <0.01). The adult sex ratio of *C. ornata* was strongly male biased and in contrast with other studies suggesting that parental care poses sex-specific mortality on the guarding parent (Smith & Wootton, 1995). This can be attributed to the absence of any natural predator of knifefish in Laguna de Bay and to current retrieval methods employed by local fisherfolks, which differentially target individuals in open waters which are predominantly females. Whereas, due to their parental behavior male individuals were concentrated in their nesting sites (vertical bamboo poles of fish pens and cages) specially during incubation and early developmental stages of their larvae. This instinctive behavior includes construction of nest, constant fanning and defending of eggs and larvae from any potential predator.

The same parental behavior which increases the survival of knifefish embryos can be exploited for control measures (e.g. manual retrieval, electrofishing and chemical control). Putting more fishing pressure on the guarding parent will result in higher parental care cost that has effects on its future reproduction, health and survival. Increased pressure during guarding will result to male energetic content decline and to compensate, male also increases its daily consumption (Steinhart *et al*., 2004). When daily consumption of guarding male increased, their departure frequency also increased, spending more time away from their nests than when males were in better condition (less fishing or predatory pressure). Natural predators of fish eggs from the lake like *A.manilensis* (Manila sea catfish) and *G. giuris* (white goby) can seize this opportunity to consume offspring from unguarded nests. After predation, damaged broods should receive less care and may be more likely to be abandoned by the nest-guarding male than intact or large broods (Sargent, 1988).

Sexual size dimorphism

Females have significant longer mean total length (60.54 cm) over males (46.97 cm, Mann-Whitney test, W = 678, P = <0.001). Size difference was found among male and female knifefish individuals. Larger body size of female knifefish may have implication to their reproductive success. Males appear to demonstrate a preference for mating with larger females in some species (Wootton & Smith, 2014). Male preference for larger females may be linked to the general correlation between female fecundity and body size in fishes (Wootton, 1998), which means that directing courtship at larger females may be more profitable. Smaller body size of male individuals can be mainly attributed to their parental care behavior. In general, male body condition declines from the start (i.e., guarding unhatched embryos) to finish (i.e., guarding juveniles) of parental care, while the amount of energy spent on guarding is positively correlated with number of potential predators, size and age of the brood (Steinhart *et al*., 2004). Physical removals should give special consideration for the female biased sexual size dimorphism of *C. ornata* because the effectiveness of these method (e.g., intensive gill netting) may vary with different body size of the target fishes. (Britton *et al*., 2011). Thus, we suggest that the body size of the smaller *C. ornata* males is the determinant for minimal mesh size of nets used in the physical removals of *C. ornata.*

CONCLUSION

Our results have determined several aspects of *C. ornata* reproduction in Laguna de Bay that are of immense value with regards to its population control. Consequently, the approaches taken in the management of this species should incorporate these findings. First, the physical removals using nets should be redoubled from December to March based on their spawning cycles. Second, the body size of the smaller *C. ornata* males is the determinant for minimal mesh size of nets used in the physical removals. Third, the efficiency of the control method should be improved (e.g., physical removals should be conducted mainly in open waters of Laguna de Bay for female *C. ornata*, but in aquaculture areas for male *C. ornata,* because the two sexes show a strong habitat segregation in the lake during spawning season as driven by parental care behavior of males). Fourth, since the presently undertaken control operations are only physical removals, application of synergistic remediation may be a complementary strategy (e.g., chemical eradication using rotenone (Ling, 2002), sex pheromones (Li *et al*., 2002), electrofishing (Dolan & Miranda, 2004) and light trapping (Meekan *et al*., 2001)). In addition, further investigations on life history traits and ecological impacts are imperative for a comprehensive management strategy of these invasive fish species.

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