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Reproduction, sexual maturity, and spawning ecology of Talang, *Scomberoides commersonnianus,* and Needlescaled queenfish, *Scomberoides tol* in Pakistan

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Abstract

An analysis of sexual pattern, spawning ecology, and size at sexual maturity in two queenfishes (Talang queenfish, *Scomberoides commersonnianus* and needlescaled queenfish, *S. tol*) was made. Fish samples were collected monthly by gillnet and trawls from the northern Arabian Sea coast of Pakistan from July 2013 to June 2014. The gonad maturity was categorized in six developmental stages based on visual analysis (colour, morphology) and histological screening of the gonads. The males of Talang queenfish dominated the population with a ratio of 1:0.4 (♂ to ♀) whereas females were higher (1:1.6) in needlescaled queenfish population. Talang queenfish was reproductively active during summer with a peak in June and winter spawning season, which appeared from November to February. Needlescaled queenfish is repeatedly spawner, appeared to be in spawning in the summer and fall. Estimated size-at-sexual-maturity for Talang and needlescaled queenfish suggests that the minimum legal length (MLL) should not be set lower than 35, 38 cm TL respectively. This conservative MLL, equal to or more than a length at functional maturity of both queenfish species would safeguard immature individuals until they reach a size at which they can contribute to the reproductive capacity of the populations in the northern Arabian Sea coast.

Keywords: Age and length-at-sexual maturity, Legal landing size, Queenfishes, Northern Arabian Sea.

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Introduction

Talang queenfish, *Scomberoides commersonnianus* Lacepède, 1801 and needlescaled queenfish, *Scomberoides tol* (Cuvier, 1832), family Carangidae, are exploited as an important commercial fish species occurring in inshore and offshore waters of Pakistan. These species constitute a huge fraction in catches in terms of biomass to overall commercial fishery landings. Among thirty three species talang queenfish contribution is very high by means of weight and number, unambiguously more valued species of the family Carangidae in Pakistan (Qamar *et al*., 2016). A reproductive pattern in fishes is as diverse as their adoptions to numerous aquatic environments. Such types of diversity may concern sexuality, spawning seasonality, behavior, sensitivity to environmental factors, and particular gametogenic processes (Jalabert, 2005). Studying size at sexual maturity is an essential parameter to understand the impact of fishing mortality on spawning stocks and the optimum fishery level (Polovina, 1987). In Australia, Griffith *et al*. (2005) reported biological parameters which included the reproduction of Talang queenfish, however, the life history characteristics of the genus *Scomberoides* remains largely unknown despite its huge commercial importance. In Pakistan, Panhwar *et al*. (2014) reported stocks of *S. commersonnianus* and Qamar *et al*. (2016) described the population structure of *S. tol* and *Megalaspis cordyla*, and also feeding habits in *M. cordyla*. The multi mood of oocytes in a shad species *Hilsa kelee* has been reported by Panhwar *et al*. (2012) in Pakistan. Knowing about the sexual cycle, reproductive potential, and sizes of sexual maturity of two queenfishes are essential prerequisites for effective conservation as well as formulating management strategies, and sustainable use of high ecological and economic species in the northern Arabian Sea coast, which are discussed.

Materials and methods

Sample collection and laboratory handling

Fish samples of *Scomberoides commersonnianus* and *Scomberoides tol* were collected at monthly intervals from July 2013 to June 2014 from commercial catches. Specimens were mainly caught with gillnets and trawls on the northern Arabian Sea (Fig. 1). Fresh specimens were transported to the Fishery Biology Laboratory, Center of Excellence in Marine Biology of the University of Karachi. Standard morphometric measurements were taken (cm) as well as total body and gonad weight (gram), and ovary colour. Initial sex assessment was made after dissecting each following method described by Brown-Peterson *et al*. (2011).

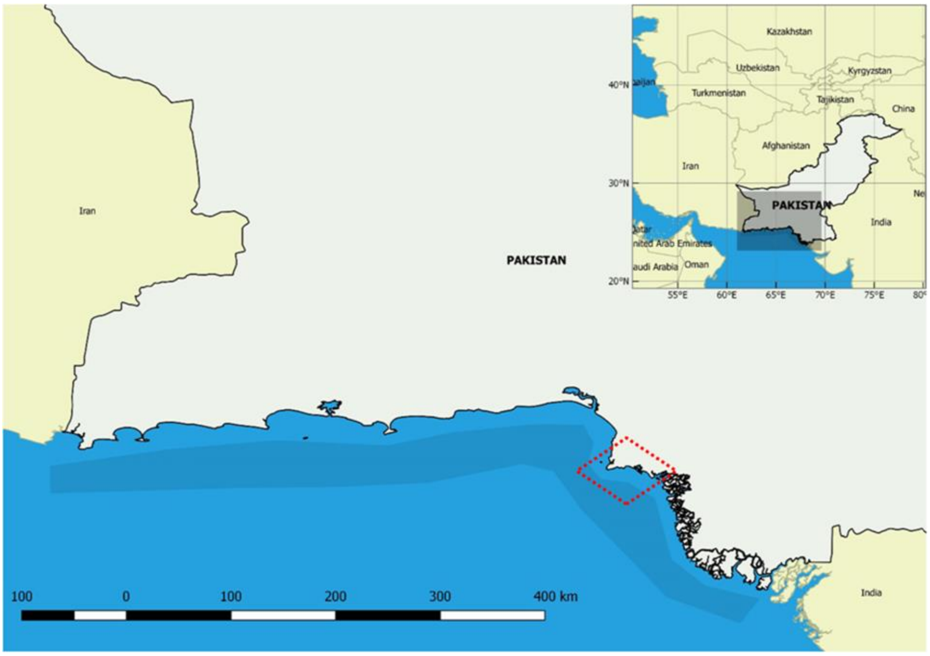

Figure 1: *Red dotted area* indicating main fish landing site (Karachi Fish Harbour) Paksitan

Gonad histology, sex ratios, and GS index

Macroscopic analysis of gonad stages was based on colour and presence of granulated material, whereas microscopic stages were also evaluated to determine the sex and developmental stage. The histological sections of gonadal material were made from tissues of 438 Talang queenfish, *S. commersonnianus* and 353 of needlescaled queenfish, *S. tol*. The method for assessing gametogenesis and early life history stages closely followed those of Brown-Peterson *et al*. (2011).

Sex determination was carried out using the histological examination of each gonad. A central piece of the gonad was fixed in Davidson's fixative for 48 hours (Shaw and Battle, 1957). Subsequently, tissues were embedded in paraffin, sectioned at 5-7 µm using a rotary microtome, stained with Delafield's hematoxylin, and counterstained with eosin (Humason, 1967). The monthly sex ratio (males to females) was estimated for total samples and was statistically tested for significant deviations from the expected 1:1 ratio with Pearson's chi-square goodness-of-fit test. The degree of gonad maturation in males and females was identified and classified closely following those of Brown-Peterson *et al*. (2011). The monthly gonadosomatic index was calculated separately for each sex:

$$GSI = \frac{Gonad\ weight}{Gonad\ free\ body\ weight} \times 100$$

whereas gonad free body weight was calculated by subtracting gonad (ovary/testes) weight from body weight. Spawning seasonality in both sexes was determined by the monthly gonad weight (GSI) and also based on the histological characteristics of the gonads during the maturation cycle. The spawning time and length period for each species was the period between the first and last collection of the fish specimens containing mature gonads.

Length at sexual maturity ($L_{50\%}$)

The size at first maturity was estimated by calculating proportion of mature males and females using total length each size class at an interval of 2 cm using equation: ($P_i=S_i/T_i$) T (Quinn and Deriso, 1999) where Si = number of sexually mature individuals of size class i; Ti = Total number of individuals observed of size class i. The logistic curve was represented by the equation: $P = \frac{1}{1+e^{-r(L-L_{50})}}$ where P=the proportion of mature fish per size class, L=average total length against each size classes; r = slope of curve; L_{50}= total length at 50% maturity (Hunter *et al.*, 1989).

Age at sexual maturity

The parameters of age and growth were calculated from length frequency data sets with the von Bertalanffy growth model equation:

$$L(t) = L_{\infty}\{1 - \exp\left[-K * (t - t_0)\right]\}$$

The age at first sexual maturity was determined as:

$$t_{50} = t_0 - \frac{1}{K}\log\frac{L\infty - L_{50}}{L\infty}$$ (McPherson, 1965)

Where: t = age, L = asymptotic length where fish size does not grow and K=growth coefficient.

Life span

Maximum age of sampled fish (t_{max})can be calculated by following (West, 1990), using the estimated parameters of the von Bertalanffy growth function: $t_{max} = t_0 + (3 / K)$.

Fecundity assessment

The relationship between fork total and gonad free body weight was described by the function $F = aL^b$, where F = number of eggs in thousands, L = length in centimeters, parameter *a* and *b* constants. The value of parameters *a* and *b* were estimated by fitting linear function F = Log a + b Log L. The fecundity data were tested for normality and homogeneity with the coefficient of variance (CV).

Temperature and precipitation

An average temperature and precipitation data was acquired from the SUPARCO / Meteriological department Karachi (Fig. 4).

Results

Gonad histology, sex ratio and GSI

Talang queenfish

Macroscopic examination of reproductive inactive and frozen fishes was difficult, so histology was used for confirmation. Histological sections of gonadal material were made from the tissues of 438 individuals of *S. commersonnianus*. Males constituted a significantly greater proportion of the population. Males were more susceptible to fishing pressure and mortality. The sex ratio was significantly different 1:0.4 males to females calculated for the total samples and was statistically tested with Chi-square analysis x^2=64.44, df =1, and p<0.005 (Fig. 2a).

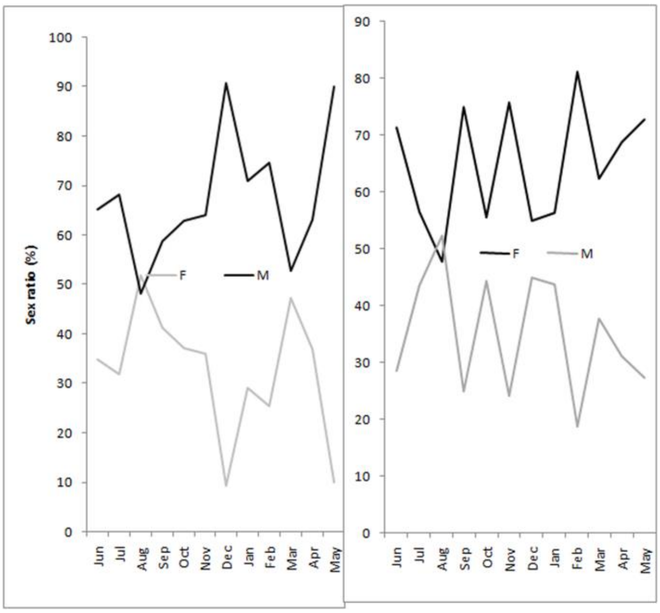

Figure 2: Description of male and female ratio in twelve sampling months (a) Talang and (b) needlescaled queenfishes sampled from July 2013 to June 2014, northern Arabian sea.

GSI of females begins to increase from March to July, which indicates the initiation of spawning during monsoon (Fig. 3). Actively spawning oocytes had yolk granules and clear oil vacuoles while spermatozoa were crammed in patches (colour plate). Another minor peak was started from November to January during post monsoon post-monsoon. The spawning in both sexes was determined by an assessment of GSI and microscopic gonad characteristics. It is interpretable from GS index that spawning season initiates in March and lasts in July showing a peak in June (1.44% F, 0.75% M) with another season appeared from November to February with a peak in December (0.50%F, 0.45% M) (Fig. 3a). Based on the microscopic gonad characteristic male and females in the late ripe, ripe, and running ripe were considered as in spawning. The GS index of both sexes rises steadily from immature to late ripe gonads due to the presence of increasing percentages of spermatozoa and oil granules and falls gradually during running to spent stages due to shedding of eggs and sperms.

Needlescaled queenfish

The sex ratio 1:1.6estimated for males to female of *Scomberoides tol* indicated that female was significantly in greater proportion and was not statistically significant (x^2=36.787, p<0.10) calculated in this study (Fig. 2b). Among the entire sampled population size, class ranged from 24-30 was found to be dominated. The monthly GS index was used to estimate temporal variation in the males and females

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spawning (Fig. 3b). The GSI estimated for females and males showed peaked in June due to the presence of active reproductive phase, while the lowest value calculated in February determining the presence of residuals of oocytes and sperms respectively.

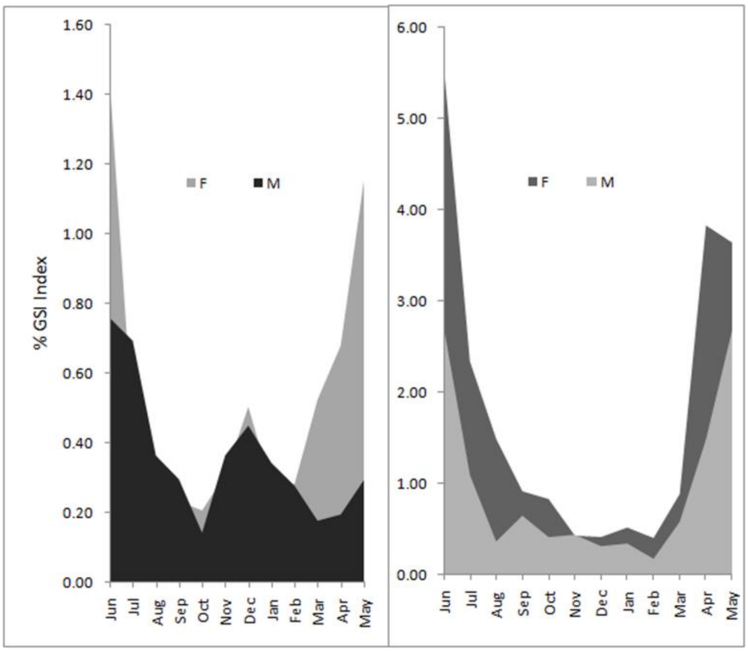

Figure 3: Temporal variations in gonadosomatic index in terms of male and female of Talang queenfish (a) and needlescaled queenfish (b) from northern Arabian Sea.

The calculated percentage GS index in six developmental stages in females are the immature stage (I) 0.25 g; stage early ripening (II) 0.52 g; late ripe stage (III) 1.10 g, ripe stage (IV) 3.51 g and spent 0.50 g. The calculated percentage of GS index in six developmental stages in males are immature stage (I) 0.23%; early ripe stage (II) 0.36; late ripening stage (III) 0.43; ripe stage (IV) 1.66% and spent (V) 0.36% was calculated (Fig. 4).

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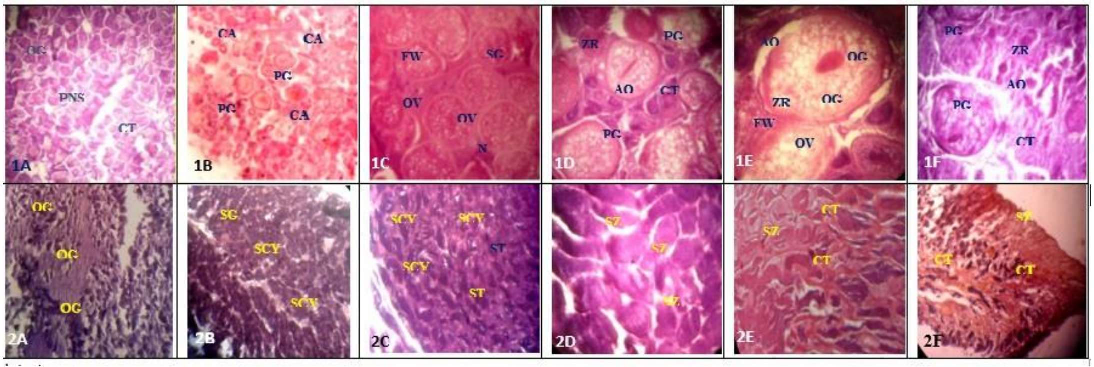

Plate I

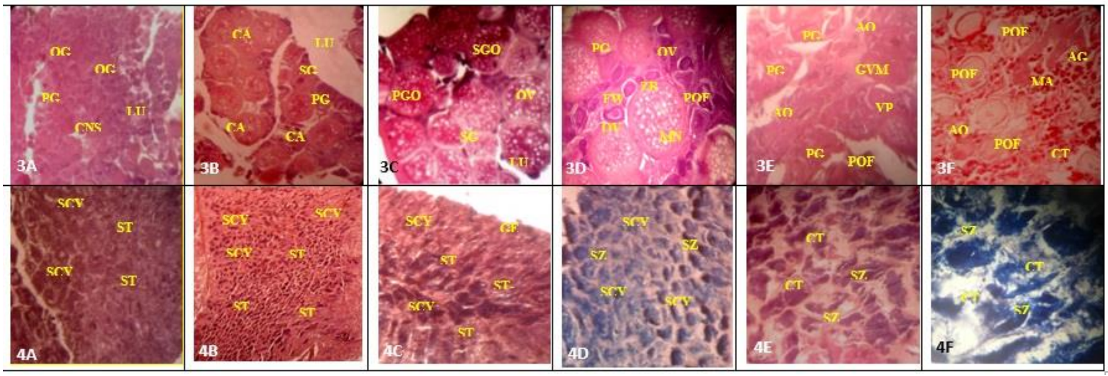

Plate II

Figure 4: Photomicrographs for different developmental stages Plate I (1A) female (2A) Male, Plate II (3A) female (4A) male of Talang queenfish and needlescaled queenfish, respectively (SG=spermatogonia, SCY= spermatocyst, ST=spermatids, GE= germinal epithelium, Lu= Lumen, CT= connective tissue, PG= primary growth oocyte, OG= oogonia, CA= cortical alveoli, N= nucleus, POF=post ovulatory follicle, GVM= germinal vesicle migrations, AO=atretic oocyte, MA=Macrophages aggregates, CT= connective tissues, OV=oil vacuoles,). Photograph @ 50 micron.

Gonad developmental in relation to the monsoon oscillation

Oscillation of monsoon that is a significant characteristic of the northern Arabian Sea, enhance spawning efficacy of both of the species, the variation of rain and temperature hike during monsoon and off monsoon are depicted in (Fig. 5).

Talang queenfish

The microscopic evaluation suggests that oogenesis initiates in December and lasts up to March when most females found immature (1 stage) with a peak occurring in January followed by August to October. The early ripe (II stage) females distingue from the presence of primary oocytes and cortical alveoli appeared from the end of March up to April. The late ripe and ripe (III and IV stage) females were identified with oil droplets and zona radiata noted from May to August peaked in June. The females in running ripe (V stage) appeared in the population in November, December and April characterized by the postovulatory follicle and atretic oocytes.

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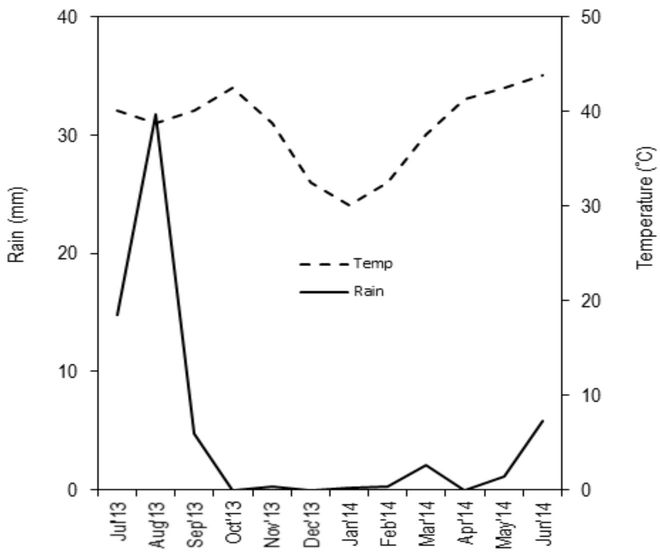

Figure 5: Average temperature and precipitation recorded in the respective year 2013-2014, northern Arabian Sea coast of Pakistan.

The spent (VI stage) females were identified with recrudescence gonads observed in various months such as February, April, May, and November to December. The process of spermatogenesis initiates when immature (I stage) appeared in December to February and peaked in January, the immature males were also observed in October. The individuals in the early ripe (II stage) were recorded in March and May. These were peaked in March with clearly seen abundant spermatocytes. Such conditions were reappeared in September and December. The late ripe and ripe (III and IV stage) was initiated from April to June with the highest of individuals in the ripe stage captured in June when most of the spermatozoa packed in the lumen. The running ripe (V stage) males observed in November with centrally degenerated germinal The individuals in the running ripe stage were appeared in the population epithelium and the resting/spent (VI stage) noted with residuals of unshaded spermatozoa and expansion of connective tissues in November to December and their peak (38%) found in February.

Needlescaled queenfish

The microscopic gonad stages were categorized in six developmental stages: I, immature; II, early ripe; III, late ripe; IV, ripe stage; V, running and VI, recently spent stage. Most of the immature females with thin translucent ovary observed in November and very few in December. The individuals of the early ripe stage were in considerable number with an abundance of secondary growth oocytes in February and the lowest in May. Hundred percent of females with yolk plates were in a late ripe stage in June and fewer in October.

from August to December with a peak in September and rarely seen in

November. The individuals went in spent condition from July, and these individuals significantly noted with brown bodies and atretic oocytes in August and October. The males in the immature stage noted in various months, and their peak found in March with few spermatogonia. The individuals entered in the next developmental stage (early ripening) noted in a substantial number in February. The individuals in the late ripe stage with spermatids in patches observed from March to May with the highest percentages in May. The ripe individuals appeared in May and peaked in June, nevertheless few males in a ripe condition were also noted in November. The individuals in the running ripe stage with proliferated spermatozoa found in July to December with the highest recorded in September. The spent male gonad was peaked in December with abundant connective tissues.

Length and age at sexual maturity

Talang queenfish

The males and females attained length at first maturity in 35 and 39 cm of total length respectively obtain from the logistic fit model, whereas males matured earlier than females. The age-at-maturity and t_{max} for combined sexes was 4.4 and 11.8 years respectively (Fig. 6).

Needlescaled queenfish

The males and females attained sexual maturity between the length of (38-39 cm) total length and age 5.13 years. The t_{max} was calculated 7.9 years for both sexes (Fig. 6).

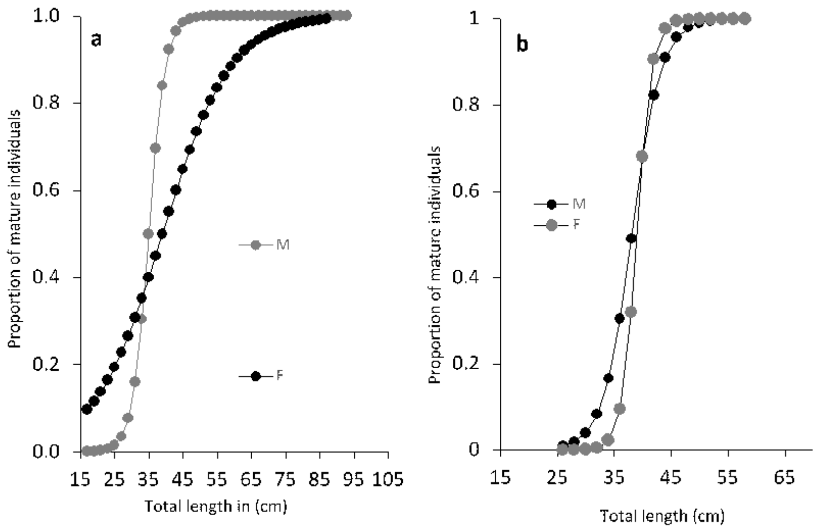

Figure 6: Sigmoid curve fitted for both sexes of talang, *S. commersonnianus* and needlescaled queenfish, *S. tol* sampled in 2013-2014, northern Arabian Sea (L_{50} = 35, 39 for females and males, respectively age of maturity for combined sexes 4.4, t_{max} = 11.8 years estimated for talang queenfish; L_{50} = 38.1, 39 male and female respectively, age at maturity 5.13 year and t_{max}= 7.9 year).

Reproductive potential (Fecundity assessment)

Talang queenfish

The reproductive potential (fecundity) was estimated from ten ripe females ranging from 57.5-112 cm TL and weighing 1140-10500 g,respectively. The number of eggs counted was 95250-594783 CV= 0.590 with an average number of 398063 of eggs counted from females considered in the spawning stage. Linear regression was applied to test the relationships between fecundity and fish body variables such as fork length, and gonad-free body weight is interpretable from the coefficient of determination R^2 values (Tables 1 and 2).

Needlescaled queenfish

Assessment of fecundity was made from sixteen ripe females of IV-V stages, measuring 38-68 cm TL and weighing 600-1700 g chosen in different months.

Table 1: Regression estimation for batch fecundity expressed as number of eggs/g ovary-free body weight and FL for two Carangids in the Northern Arabian Sea coast.

Species	Equation	Intercept (a)	Slope (b)	SE of the estimate	R^2
S. commersonnianus	F= a+b FL	1.844	2.039	0.200	0.716
	F= a+b GFBW	0.252	4.566	0.328	0.270
S. tol	F= a+b FL	1.773	2.440	0.223	0.200
	F= a+b GFBW	2.452	1.161	0.072	0.821

Table 2: The reproductive potential (fecundity) estimated for two Carangid species (Stage IV-V) in this study (CV= coefficient of variation).

Name of the species	Total length (Range in cm)	Body weight (Range in g)	Min and Max eggs (average)	CV
S. commersonnianus	57.5−112	1140−10500	95250±594783 (398063)	0.590
S. tol	38−68	600−1700	111974±417043 (233883)	0.454

The counted number of eggs ranged 111974±417043 (CV=0.454), and the average number of eggs counted for each ripe fish was 233,883. The relationship between fecundity and fork length and gonad-free body weight was Log_{10} F=1.773+2.440 Log_{10} FL, R^2=0.200; Log_{10} F=-1.161+2.452 Log_{10} OFBW, R^2 = 0.821 is interpretable from the coefficient of determination (Tables 1 and 2).

Length-at-age

The fitted logistic growth curves for Talang queenfish and needlescaled queenfishes were estimated for both sexes and relatively departed from each other (Fig. 7).

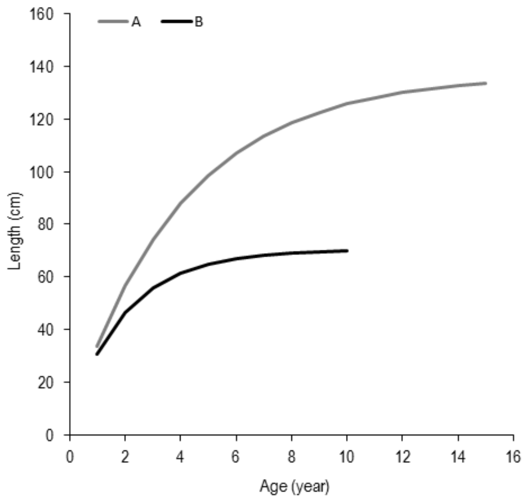

Figure 7: Length-at-age data and logistic growth model estimated for Talang queenfish (A) and needlescaled queenfish (B).

Discussion

Annual gonad determination of two queenfishes demonstrated that both species are repeatedly spawners that appeared with certain peaks in the northern Arabian Sea coast. The sex ratio *Scomberoides commersonnianus* appeared to be male-biased, the reason for this biasness is unclear. Nevertheless, biased sex ratios might be due to sex-specific migration, differences in growth or mortality rates (Panhwar et al., 2012). The prevalence of adult males in catches was different to that from observations made by Griffiths *et al*. (2005) who collected 50% males and female of *S. commersonnianus* in northern Australia. The dominance of one sex to other could be related to the differential growth and mortality (Kaiser, 1973) or may be the earlier maturation of both sexes. Females typically spend noticeably high effort in reproductive development comparing to males (Gabr *et al*., 1998). Therefore, ovaries reflecting the duration of fish spawning activity is a general hypothesis. Besides, lipid constituents in gonads also influence the maturation cycle and spawning period, which was studied in *Scomberoides lysan* from Indian waters(Norungee and Kawol, 2011).The gonad maturation in *S. tol* reached at advance stages during the summer and spring at the time when spawning takes place. Further multimode of eggs validates spawning partiality. Due to the dearth of published literature on the reproductive biology of *S. tol*, it was not possible to compare the results of this study. However, the reproductive periodicity or spawning pattern could differ in Carangid species, some Carangid shows dual reproductive pattern and some spawns once a year (Conover, 1992; Cortés, 1999). In general, the maturity could be impacted by temperature, food, and genetics influence. Our finding validates that both Talang queenfish and needlescaled queenfish are partial spawning species, primarily spawn in summer and winter observed

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in the northern Arabian Sea. Spawning season and sexual maturation are vital for estimating population dynamics and management of the species stocks (Thulasitha and Sivashanthini, 2013).Two spawning seasons (summer and winter) were observed in *S. commersonnianus*. Generally, most of the marine inhabitants in tropical seas spawn throughout the year while showed seasonal breeding (Hopper *et al.*, 1998). In this context, Weatherly and Gill (1987)stated that the spawning period in many bony fishes living in lower latitude commences earlier and may last longer, it may be related to the rate of the stress of the protracted growing season and a small winter. Koob and Callard (1999)reported from Sri Lankan water that *Scomberoides lysan* spawns twice a year with intensity occurred in September. Besides, it described that either sea temperature or photoperiod or both directly involved in reproductive synchrony. It directly related to biological production and food availability as well as plankton compositions, which ultimately influence the growth of fish. The possibility of mortality during spawning season is also high, and chances to produce atresic oocyte appeared.

Size at sexual maturity data is acquired for assessing spawning stock biomass and for locale reference points for fishing seasons and dynamics. The length and age at the first sexual maturity endorsed that needlescaled queenfish attained later than the Talang queenfish. In this context, Griffiths *et al.* (2005) reported that it is three years a little bit earlier than queenfishes occurring in the Northern Arabian Sea. Nevertheless, males often mature earlier than females. This is because the liver plays an important role in regulating reproductive cycles by stocking lipids and contributes in the process of vitellogenesis. Another reason may be due to the habitat preference of both sexes, which not gratify the maturity at the same size. On the sexual maturity, Griffiths *et al.* (2005)reported that females of *S. commersonnianus* matured as 476 mm in Australian water grew quickly in early years of the age (up to 3 years), and maximum age was calculated as 11 years. Nevertheless, growth estimation of this species is relatively fair in Pakistan (Panhwar *et al.*, 2014) than the Australian waters. Both sexes of *S. commersonnianus* sexually mature at the length of 35cm and 32cm at the age of 4.4 years, respectively. This length at maturity was contrasting with those reported on *S. lysan* (Norungee and Kawol, 2011). The Carangids are fast growing up to the age of three years than growth diminish up to their maximum life i.e. 14 yr and at the length of 120 cm TL (Griffiths *et al.*, 2005; Panhwar*et al.*, 2014).

Talang queenfish found to be more fecund than, needlescaled queenfish, egg counting from ripe females of Talang queenfish was higher than counted (Griffiths *et al.*, 2005) earlier rom Australian waters. The assessment of reproductive potential indicated that

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S. tol releases more eggs than *S. commersonnianus* such variations may be occurring due to environmental adaptation that impacts on species to species. However, due to the dearth of published literature on the reproductive potential of needlescaled queenfish, we were not able to compare.

There was no evidence of sexual dimorphism found in both queenfishes determined from histological sections of male and female reproductive organs. Hence, it appeared that both species are dioecious having two distinct sexes. Queenfishes are repeatedly spawner validated from monthly GSI oscillation and synchronous oocyte development, noted around the year. Assessment of size-at-sexual-maturity endorses that minimum landing length (MLL) should not be set lower than 32, 38 TL_{cm} for Talang and needlescaled queenfishes, respectively. This conservative MLL, equal to or more than length at functional maturity, would safeguard immature individuals until they reach a size at which can contribute to the reproductive capacity of the populations. It is expected that data and outputs of this study would greatly help in guiding management decisions if conservation strategy developed for queenfish fishery in the area.

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