Aspects of Spawning Behaviour in Five Gobiids of the Genus *Coryphopterus* (Pisces: Gobiidae) in the Caribbean Sea

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Abstract: Sand dwelling species of *Coryphopterus* live in shared habitats and were reported to have a similar ecology. Species lineages within the western Atlantic *Coryphopterus* have recently been reconciled; fish which according to the common keys had formerly been identified as *Coryphopterus glaucofraenum* could in fact be *C. glaucofraenum, C. tortugae* or *C. venezuelae* and can genetically be clearly separated into different clades. Based on the new taxonomic key, ecological data for five Coryphopterus species are provided; size-sex distribution, number of eggs deposited, inter spawning intervals and courtship behaviour of *Coryphopterus dicrus* Böhlke and Robins, *Coryphopterus eidolon* Böhlke and Robins, *Coryphopterus thrix* Böhlke and Robins, *Coryphopterus tortugae* Jordan, and *Coryphopterus venezuelae* Cervigón, were examined in Curacao, Netherland Antilles. For the first time ecological data on *C. tortugae* and *C. venezuelae* are provided, which had been previously questioned as distinct species from *Coryphopterus glaucofraenum.*

In all species males reached a greater total length than females. Clutch sizes varied between 423 – 5872 eggs and inter-spawning intervals were between 5 – 14 days; no preferences for spawning at a particular lunar phase was found.

Gobiids have evolved many different ways of reproduction, for example monogamy, hermaphroditism, sneaking into other nests or bi-directional sex change [1-11]. Number of eggs and fecundity varies widely among and within species, ranging from less than 100 eggs in the small tropical marine goby *Eviota lacrimae* to over 500 000 eggs in the large tropical freshwater goby *Awaous guamensis*, most of these observations have been based on ovary dissections with only a few studies conducted in the field [12, 13]. The advantage of experiments conducted in aquaria is that clutch sizes can be documented from a single female, as opposed to natural conditions where the clutch may be produced by several females and be in different stages of development [14].

Coryphopterus species have been reported to have a sequential (protogynous) hermaphroditic life history [12]. Detailed studies in the genus *Coryphopterus* have suggested that most species in that genus, including *C. eidolon, C. dicrus* and *C. thrix* are protogynous hermaphrodites, no data on reproduction have been available for *C. tortugae* or *C. venezuelae* [5, 6, 13]. However, some of the previously recorded data might not be accurate as the identification of western Atlantic *Coryphopterus* species has historically been difficult because of morphological similarity among some species and uncertainty regarding the number of valid species. Baldwin *et al.* [15] have recently reconciled western Atlantic *Coryphopterus* species with genetic lineages to determine the number of valid species and provided a revised taxonomic key.

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The five investigated species in this study are of similar adult size, and are found in coral reef environments; *C. eidolon, C. glaucofraenum, C. tortugae* and *C. venezuelae* live associated with sand patches in between coral outcrops, whereas *C. thrix* and *C. dicrus* are more associated with algae covered coral rocks at the edge of the sandy areas. The aim of this study was to investigate their strategies in reproduction, describe in more detail their ecology, and contribute to the determination of specific factors causing variations within these closely related species.

MATERIALS AND METHODOLOGY

All studies were performed between April and November 2005, in Curacao, Netherland Antilles. The sample location was the reef south of the Curacao Sea Aquarium (GPS coordinates + 12.08371 - 68.89645). Fish were collected with Quinaldine (2% in alcohol) and hand nets. Species were identified according to Baldwin *et al.* [15]. In total 198 fish of different sizes of the five species were collected; 36 *C. dicrus*; 56 *C. eidolon*, 38 *C. thrix*, 7 *C. tortugae*, and 67 *C. venezuelae*. The fish were measured (total length, TL) with a dial calliper (Draper 4817P) to the nearest 0.1 cm and sex determined by examining genital papillae using a binocular microscope [3, 4, 16]. The fish were placed into aquaria (200 litres) in pairs for reproductive studies. The tanks were supplied with a continuous flow of fresh sea water and kept at an ambient temperature between 28° and 32°C. They were exposed to natural light on the outside of the Curacao Sea Aquarium. The bottom of the aquarium was filled with sand and segments of polyvinyl chloride (PVC) pipes between 3 and 5 cm in diameter and 8 to 10 cm in length, which provided shelter and breeding substrate for the fish. Five pairs of *Coryphopterus eidolon*, four of *C. thrix* and three of *C.*

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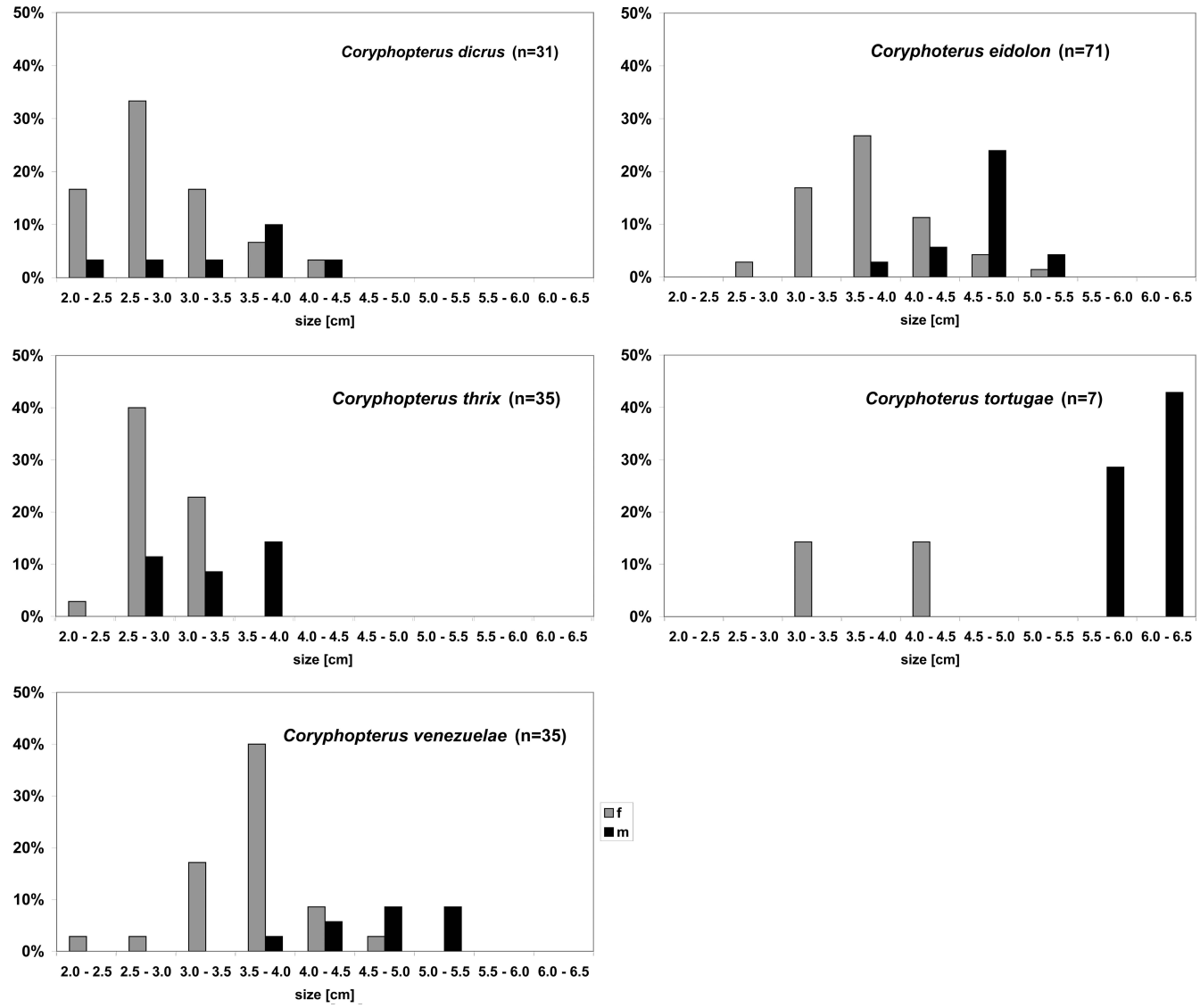

Fig. (1). Size classes – sex distribution in *Coryphopterus* spp. Significant differences were found between male and female body sizes in *C. venezuelae* and C. *tortugae*. Species identified by molecular studies.

dicrus were used in the study, as well as nine pairs of *Coryphopterus* cf *glaucofraenum*; either *C. glaucofraenum, C. tortugae or C. venezuelae* (see results for species identification problem). Females used in the spawning experiments were 3.0 - 4.4 cm in total length. Spawning was observed in all species. Each PVC pipe was lined with an acetate sheet to allow easy removal of the eggs and clutch size determination [14]. The fish were fed live brine shrimps three times a day and aquaria checked for eggs. After the eggs were laid, they were removed from the PVC pipe, on the acetate sheet, photographed with a digital camera (Olympus C-5060) and counted on the photograph. Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago). Paired t-tests were used to determine significant differences in number of eggs deposited by species and between body sizes of males and females. For differences in body size of males and females the p value was considered significant if less than 0.05, the Bonferroni correction was used for correcting the values of multiple paired t-tests for the number of eggs between the four species and therefore results considered significant at p-values less than 0.0125.

RESULTS

For *C. dicrus* 36 individuals were examined (in total length: TL 1.0 - 4.3 cm), for *C. eidolon* 56 individuals (TL 1.3 – 5.4 cm), for *C. thrix* 38 individuals (TL 1.0 – 3.9 cm), for *C. tortugae* 7 individuals (TL 3.2 – 6.2 cm) and for *C. venezuelae* 56 individuals (TL 0.9 – 5.1 cm); the later two had originally been identified as *Coryphopterus glaucofraenum* according to the standard keys [17-19], but ge-

Table 1. Number (no.) of Egg Clutches Observed, Range and Mean Number of Eggs, Interval Between Two Consecutive Spawning Events. Numbers of Eggs Deposited are Highly Variable Among Individuals and Species

	No. of Clutches	No. of Eggs Range	No. of Eggs Mean ± SD	Days Between Spawning
C. dicrus	7	423 – 1265	829 ± 272	5 ± 2
C. eidolon	23	629 – 3371	1734 ± 716	8 ± 5
C. thrix	6	625 – 1401	858 ± 288	12 ± 2
*C.*cf *glaucofraenum*	25	1407 - 5872	3314 ± 1225	9 ± 4

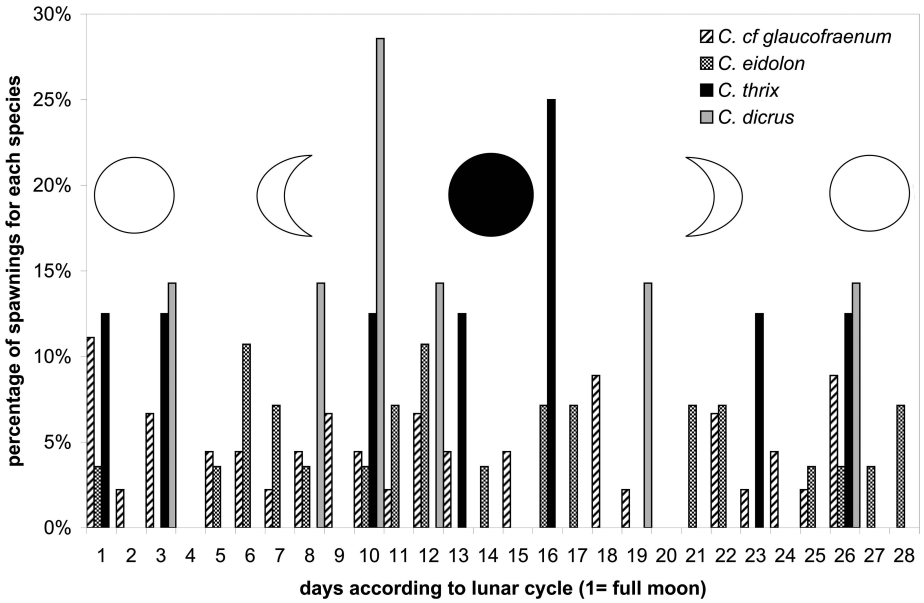

Fig. (2). Distribution of spawning in relation to lunar cycle for *Coryphopterus* spp. Spawning events occurred randomly during the lunar cycle (full moon = 1).

netic analysis and comparison with the reference sequences provided by Baldwin *et al.* revealed that some could be assigned to *C. tortugae* and *C. venezuelae* [15]. The nine pair of fish which were identified as *C. glaucofraenum* and used for spawning experiments could not be re-identified, but due to a large set of data from these fish, the results are included in the present paper and referred to as *C.* cf *glaucofraenum*. The same term is used when referring to other authors and their previous studies with results on *C. glaucofraenum*, as the fish identity is now questionable.

Total length and sex are presented in Fig. (**1**): In this study males in all *Coryphopterus* species attained a greater length than females, differences between male and female body sizes did show significant differences in body length between males and females in *C. tortugae* and *C. venezuelae* (CI 95 %). The total length (TL) at which genital papillae could visually be differentiated was similar for all species; 2.2 cm in *C. dicrus*, 2.7 cm for *C. eidolon*, 2.3 cm for *C. thrix*, 3.2 cm for *C. tortugae* and 2.4 cm for *C. venezuelae*. Data on number of eggs and intervals between spawning events for *C. dicrus*, *C. eidolon*, *C. thrix* and *C.* cf *glaucofraenum* are summarized in Table **1**, a t-test with a Bonferroni correction (CI 98.75%) resulted in no significant differences in number of eggs between the species. There was no visual evidence for a strong lunar spawning pattern: *C. dicrus* and *C. thrix* appeared to spawn preferentially around new moon, but a larger data set is needed to confirm if a preference exists (Fig. **2**). No evidence for a relationship between female body length and the number of eggs laid was observed (Table **2**); but this will also have to be confirmed with additional data. Courtship behaviour was observed in all species and was very similar. A detailed description for *Coryphopterus dicrus* is given: The male swam towards the female with very fast movements of all fins. After that, he remained inside the PVC tube most of the time, while the female remained in front of it or inside. The male's colouration changed: ventral fins becoming a dark grey colour, the transparent anal fins showing a silvery-bluish margin. The male then moved all his fins while swimming in and out of the tube. The female moved close to the entrance of the tube, positioning herself at a 90° angle to the front of the tube, and hence displaying her lateral side to the male. The male continued swimming in and out of the tube, rapidly moving all fins, except his caudal fin. The female started swimming in and out of the tube repeatedly; the male was simultaneously swimming around and towards her, exhibiting an intense colour display of blue and yellow on its body and all of its fins. The female maintained her natural colouration. In both fish the genital papilla was erect, swollen and clearly visible. The male continued to display and swim in circles towards

Table 2. Total Length (TL) of Females and Number of Eggs Observed in Each Spawning Event. There is no Visual Evidence of any Relation Between Female Body Length and Number of Eggs

	TL [cm]	1st Spawning	2nd Spawning	3rd Spawning	4th Spawning	5th Spawning
C. dicrus	3.8	830	-	-	-	-
C. eidolon	4.4	1110	629	-	-	-
C. thrix	-	-	-	-	-	-
*C.*cf *glaucofraenum*	4.1	3336	3478	-	-	-
*C.*cf *glaucofraenum*	3.5	2211	2453	2124	1407	-
*C.*cf *glaucofraenum*	3.0	2886	2864	2836	-	-
*C.*cf *glaucofraenum*	4.2	4856	4199	3537	5872	5179
*C.*cf *glaucofraenum*	4.1	1564	1736	-	-	-
*C.*cf *glaucofraenum*	4.1	1930	4664	-	-	-

the female, moving his tail fin rapidly. During another spawning event a male was observed displaying his body laterally and widely opening his mouth. The female entered and exited the tube a number of times; whenever the male swam towards her, she turned away, got closer to the tube, and finally entered backwards.

Spawning was observed several times for each of the *Coryphopterus* species. In all events, the female deposited eggs, in a single layer, on the ceiling and lateral walls of the PVC pipe, while the male swam next to her, fertilizing the eggs. A detailed description of their eggs, attaching structure and developing larvae has been given by Kramer and Patzner [20].

DISCUSSION

Spawning and reproductive behaviour in *Coryphopterus* species is similar to that described for other gobiid species: the female lays a batch of eggs in a single layer, attached to the substrate while the male fertilizes them [14, 20-22]. Cole [6] states that protogyny appears to be widespread, if not universal in the genus *Coryphopterus*. Experimental studies within the genus *Coryphopterus* have shown the ability of *C. dicrus*, *C. eidolon*, *C.* cf *glaucofraenum* and *C. thrix* to change sex and were confirmed by histological examination of gonad structure [13]. Findings in the present study which show that mean total length of males is larger than that of females is supportive evidence for protogyny, but experimental studies and dissection of gonad tissue will be required to confirm this for *C. venezuelae* and *C. tortugae* [23].

How long the fish live and what causes them to change sex under natural conditions is not known. Their maximum size has been reported to be 5.0 cm for *C. dicrus* and *C. thrix*, 6.0 cm for *C. eidolon* [24], 7.5 cm for *C. venezuelae* [25] and 6.2 cm for *C. tortugae* (this study). For the first time data are available on the size – sex distribution of *C. tortugae* and *C. venezuelae* and results indicate that they are protogynous hermaphrodites as in other *Coryphopterus* species. The results for *C. tortugae* still have to be confirmed with a larger sample size.

Sexual maturity in *Coryphopterus* cf *glaucofraenum* has been reported by Forrester and Steele [26] as occurring between a standard length (SL) of 2.2 and 2.5 cm, they did not describe the method employed to obtain those data, but refer to unpublished data. These data are close to the total length at which we could determine if fish were male or female by their genital papillae.

It is difficult to predict the number of eggs deposited under natural conditions; they can be different from numbers obtained in the laboratory and males can guard eggs of more than one female at the same time [27].

In many gobiids of temperate and subtropical zones spawning periods occur primarily in spring/summer and last for several months with a consecutive "reproductional break" [1, 28, 29]. In the present study undertaken from April to November 2005 continuous reproduction was observed and spawning in all species appeared to occur independent of lunar phases, these results will have to be confirmed on a larger data set. Sponaugle and Cowen [28] state that there might be a connection between spawning and lunar cycle in *C.* cf *glaucofraenum*, which appears to have a peak spawning period near the moon's third quarter; their data were calculated back from larval stages. It will be necessary to determine what triggers spawning events in the field as laboratory settings can bias results. The arrangement of the stable male-female pairs do not reflect the social arrangement in the field, where dominant males probably mate with multiple females during most spawning bouts, as observed in other protogynous gobiids [27]. This will also give conclusions on the minimum inter spawning interval of 3 days, observed in the present study, which is close to the 4 - 6 days reported for other tropical, reef associated gobiids, such as *Paragobiodon echinocephalus* and *Asterroptryx semipunctata*, again from observations made in lab conditions [14, 30]. Topics for future studies remain: the variability in the number of eggs (up to 3100) between different clutches, laid by the same female; factors which determine the length of an inter spawning interval and if its length has an effect of the number of eggs deposited and under what circumstances and how frequently the fish change sex under natural conditions.

CONCLUSION

The species *C. eidolon*, *C. dicrus*, *C.* cf *glaucofraenum*, *C. thrix*, *C. tortugae* and *C. venezuelae* have very similar patterns in reproduction and results support previous findings which suggest them to be protogynous. Number of eggs deposited differ greatly between different clutches and the reason for that remains a topic for future studies. No visual evidence has been found that reproductive behaviour in captivity is related to the lunar phases or body length to the number of eggs a female deposits.

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