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CONTENTS

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Cover photograph: Pied bush (*Saxicola caprata*), see Sethi et al. (page 67).

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Song sharing in the pied bush chat (*Saxicola caprata*)

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ABSTRACT. In most oscine passerine bird species, male song is composed of different song types of which some are shared with other males. Our aim for this study was to investigate whether song sharing occurs in the pied bush chat (a tropical species with multiple song types), and if so, whether neighbouring males share significantly more song types than non-neighbouring males and whether song sharing declines with increasing distance between males. A standard song sharing index was used to compare all song types of each male with all song types of all other males. Males had a mean repertoire of 22.8±4.4 song types ranging from 17 to 30 song types. Results revealed that neighbouring males shared significantly more song types than did non-neighbouring males. Nevertheless, no two males shared all song types in their repertoires. Furthermore, we observed a significant decline in the proportion of song repertoire shared with increasing distance between males. Results confirm the presence of song sharing in the pied bush chat, which may help neighbouring males to mediate social relationships.

KEY WORDS: pied bush chat, *Saxicola caprata*, song repertoires, song sharing.

INTRODUCTION

Bird song, among the most acoustically complex of all non-human vocal communication signals (Eda-Fujiwara et al., 2006), has been attracting the attention of naturalists, zoologists and ornithologists for a long time (Darwin, 1871; Baker, 2001; Marler, 2004). In general, bird song is believed to function as a dual purpose signal aimed to attract mates and/or to repel territorial rivals (Catchpole & Slater, 2008).

The number of different song types a bird sings is referred to as song repertoire size and may range from 1 to more than 2000 song types in different bird species (Catchpole & Slater, 2008). The most widely proposed explanation for the evolution of song repertoires is that they have evolved largely because of sexual selection, with females preferring males that sing many song types (Searcy & Andersson, 1986; Andersson, 1994). The underlying idea is that the development of large repertoires is costly and only high quality males can afford it (Kiefer et al., 2006).

An alternative hypothesis is that repertoires have evolved to facilitate communication among territorial neighbouring males (Beecher et al., 1994, 2000; Hughes et al. 1998; Price & Yuan, 2011). Song contests between neighbours may include either ‘song type matching’ in which one male replies to another with the same song type (Krebs et al., 1981) or ‘repertoire matching’ in which one male replies to another with a different but shared song type (Beecher et al., 1996). Studies suggest that territorial neighbouring males use song sharing as an important strategy to address a particular rival and to signal more specific information such as arousal or readiness to escalate a contest. Therefore, by song sharing (song-type matching) a bird may reduce the need for physical battles, thus minimizing the cost and risk of such escalations (Krebs et al., 1981; Todt & Naguib, 2000). If song sharing is important in territorial contests between neighbouring males, then it could enhance male fitness by prolonging territory tenure (HUGHES et al., 2007). Furthermore, a correlation between song sharing and reproductive success has also been established for indigo bunting (*Passerina cyanea*) where males that share their single song type with a neighbour tend to be more successful in mating and in fledging young than those who do not share song (PAYNE et al., 1988). Nevertheless, to understand the adaptive significance of song sharing in any bird species, one has to first ascertain whether song sharing exists in a territorial song-bird species or not.

Song sharing varies considerably between species (RASMUSSEN & DABELSTEEN, 2002). For example, males of several bird species share song types with their neighbours (MCGREGOR, 1980; MCGREGOR & KREBS, 1989; BEECHER et al., 2000; GRIESSMANN & NAGUIB, 2002; ROGERS, 2004; KOETZ et al., 2007) while others do not (SLATER & INCE, 1982; Horn & Falls, 1988) or even share more with non-neighbours than with neighbours (GRANT & GRANT 1979; BRADLEY 1981; BORROR, 1987). Furthermore, in some species, neighbours share more than one might expect (DUFTY, 1985; MORTON, 1987) while in others, sharing falls off rapidly with distance (WILSON et al., 2000; RIVERA-GUTIERREZ et al., 2010). Intraspecific differences in song sharing have also been reported, where sedentary populations of some bird species exhibit higher song sharing while migratory populations exhibit less song sharing (KROODSMA & VERNER, 1978; EWERT & KROODSMA, 1994; NIELSEN & VEHRENCAMP, 1995; NELSON et al., 1996; HUGHES et al., 1998).

Saxicola (Family Muscicapidae) is a genus of 15 species of small passerine birds commonly known as stonechats or bushchats. There exists very little information on the singing behaviour of most species in this genus. Species studied for their basic song structure in this genus include the whinchat (*Saxicola rubetra*), stonechat (*S. torquata*) and pied bush chat (*S. caprata*) (GUETTINGER, 1984; SETHI et al., 2012a). Researchers have studied seasonal patterns of song production in the stonechat (GREIG-SMITH, 1982a), correlations between song rates and parental care in the stonechat (GREIG-SMITH, 1982b) and song repertoire sizes of the pied bush chat (SETHI et al., 2011a). Other than this, a survey of the literature reveals that we largely lack fundamental information on the singing behaviour of most species of this genus.

The pied bush chat is a tropical, sedentary and territorial songbird. It is found in open habitats including scrub, grassland and cultivated areas. It is distributed discontinuously from Transcapsia and the Indian subcontinent to south-east Asia, the Philippines, Indonesia, New Guinea and New Britain (BELL & SWAINSON, 1985; ALI & RIPLEY, 1998). In addition to using a variety of calls (SETHI et al., 2012b), males of this species intensively sing on a daily basis at dawn during the breeding season (late February to July in the study area) (ALI & RIPLEY, 1998; SETHI et al., 2012a). Males have mean (±SD) song repertoires of 22.2±6.6 song types (range = 13–29; $N = 9$ males) (SETHI et al., 2011a). Males remain on their respective territories throughout the year. Nevertheless, territorial encounters between males are only observed frequently during the breeding season (pers. obs.). Female pied bush chats have also been reported to sing (SETHI et al., 2012c). A female removal experiment in this species suggested that the presence or absence of a mate does not influence male dawn singing behaviour and males seem to direct their songs to neighbouring males (SETHI et al., 2011b). The existence of large song repertoires along with the territorial habit of two or more males that sing in a close-range vocal interaction network make the pied bush chat an excellent model to study song sharing behaviour (SETHI et al., 2011a). Nevertheless, no studies to date have examined song sharing of this species. Therefore, our aim for this study was to investigate whether song sharing occurs in this species, and if so, whether neighbouring males share significantly more song types than do non-neighbouring males and whether song sharing declines with increasing distance between males.

MATERIAL AND METHODS

This study was carried out in agricultural fields and open grounds at Haridwar (29°55′N, 78°08′E), Uttarakhand State, India during the breeding season, February to July 2013, of the pied bush chat. Songs were recorded of eight males from a color-banded population. We concentrated our study mainly on dawn singing to take advantage of the tendency of males to sing at the highest rate at this time of day (Sethi et al., 2011b). We visited each territory before dawn and recorded the focal male for the entire duration of his dawn singing bout. We always reached the study area before the male started singing and remained there until the completion of the dawn singing. Males start to sing 51.7±7.3 min before sunrise (pers. obs.) and mostly become quiet around sunrise. Thus, sunrise was used as the cutoff point defining the end of dawn singing. However, sometimes males stopped singing approximately 20 min before sunrise and engaged in an alternate activity such as foraging. In these cases, we considered the dawn chorus as finished when the male did not sing for a minimum of 5 min. Song repertoire size was defined as the number of different song types used by a male.

On most occasions, two males were recorded each morning by two observers separately using a Sennheiser ME 67 directional microphone attached to a Marantz PMD 670 portable solid-state sound recorder (D&M Holdings Inc., Kanagawa, Japan). Songs were saved to a computer as .WAV files with input sampling frequency of 24000 Hz and sample format of 16 bit. Spectrograms were prepared with Avisoft SASLab Pro 4.1 software (Specht, 2002).

The eight focal males could be divided into three groups. In the first group, males 1, 2 and 3 were a neighbouring group with male 2 settling between male 1 and male 3. In the second group, males 4 and 5 were immediate neighbours. Males 6, 7 and 8 made the third neighbouring group with male 7 settling between male 6 and male 8. These three groups were at least 2 km. apart and therefore, we believe that they were out of audible range from each other. For the estimation of song repertoires of any male, we visually studied the spectrograms of its entire

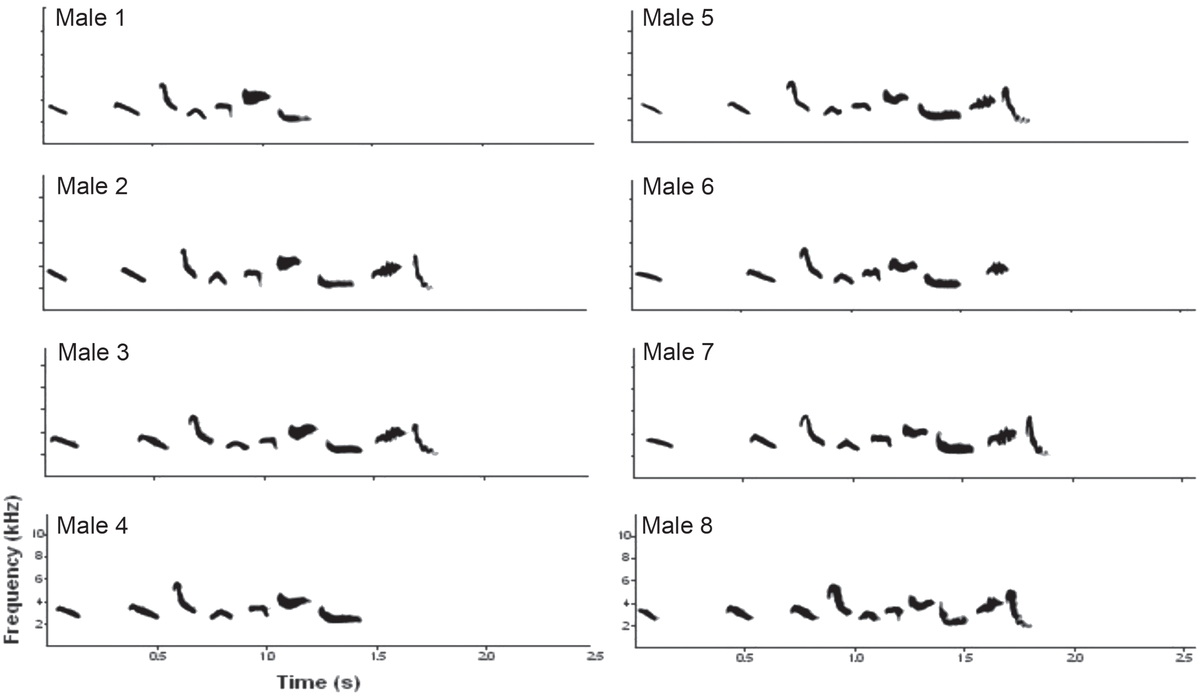

Fig. 1. – Spectrograms of a song type shared by all individuals of the pied bush chat (N=8).

song bout. For each male, a printed library of all used song types was constructed. Each song type was classified either as a new song type or as an already existing one. Each new song type was assigned a unique number. On inter- and intra-individual levels, males rarely used variants of song types, i.e. added or deleted one or two notes at the end of a song type (Figure 1). However, the variation within song types was much less than between song types, and thus, we were able to identify all song types unambiguously. Our previous observations suggested that an inspection of 320 consecutive song types of an individual male pied bush chat can generate its complete song repertoire (Sethi et al., 2011a). Therefore, we inspected around 400 song types for each male. Completeness of repertoires was verified by plotting the cumulative number of new song types against the total number of songs in the subject's repertoire. We were able to estimate the full song repertoires as the cumulative graph reached an asymptote for all males.

To assess the song sharing between males, we compared all song types of each male with all song types of the other males. In all, 28 pair-wise repertoire comparisons were made, of which 7 and 21 were made among neighbouring and non-neighbouring males respectively. Following McGregor & Krebs (1982) and Catchpole & Rowell (1993), repertoire sharing was calculated for every pair of males in the population using the standard song sharing index $S = 2Ns/(R_1+R_2)$, where Ns = the number of shared song types, and R_1 and R_2 are the repertoire sizes of the two males. This gives a song sharing index that expresses the proportion of song types shared between any two males on a scale from 0 (no song types shared) to 1 (all song types shared). A Mann-Whitney U test was used to compare the song sharing index between neighbouring and non-neighbouring males (Zar, 1999). To determine whether song sharing varies as a function of distance between two males, a Mantel test (Mantel, 1967) was used through comparing a matrix of sharing indices with a matrix of geographic distance between the males. The distance between two males was measured using a location map and calculated as the shortest land distance between their active nests.

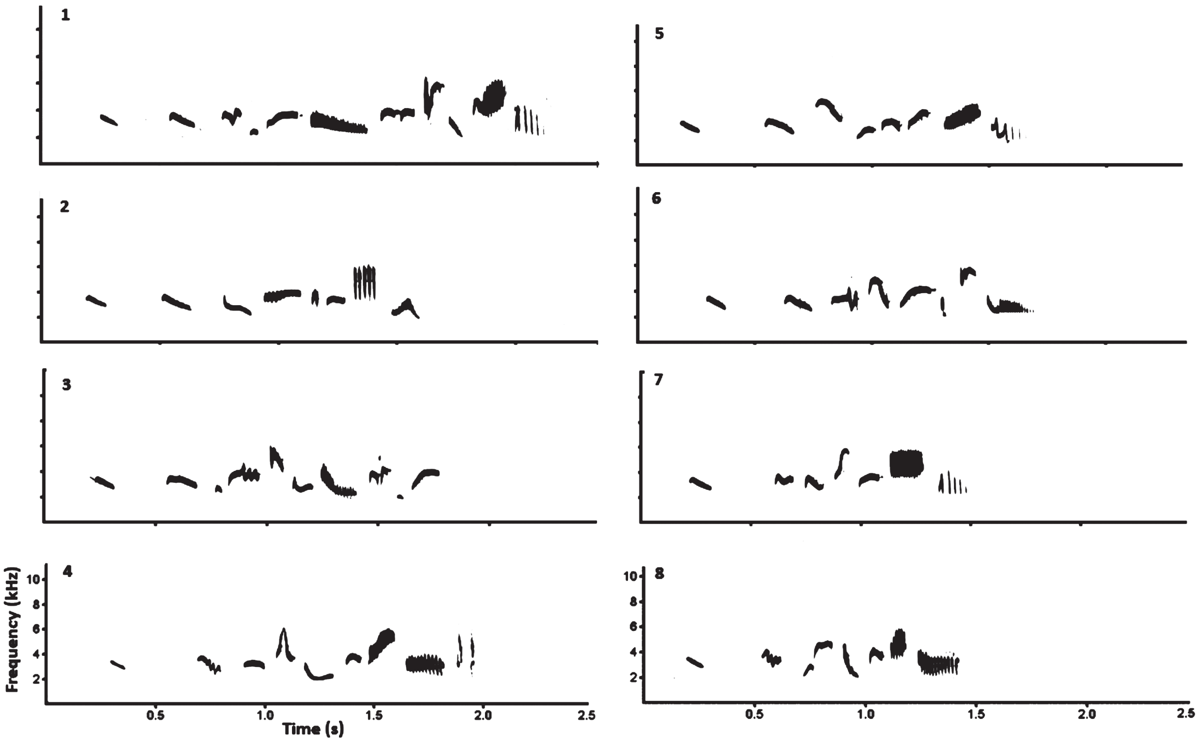

Fig. 2. – Spectrograms of eight song types (out of 20) used by male no. 1.

RESULTS

Pied Bush Chat males had a mean (±SD) repertoire of 22.8 ± 4.4 song types (N = 8 males). The song repertoire size of these males ranged from 17 to 30 song types. The spectrograms of eight song types (out of 20) delivered by male 1 are shown in Figure 2.

In this study, neighbouring males shared significantly more song types (S = 0.52±0.03; mean±SE) than did non-neighbouring males (S = 0.35±0.02) (Z = 3.18, P = 0.001). The highest and the lowest similarities of song repertoires were observed in neighbouring (S = 0.62) and non-neighbouring pairs (S = 0.22) respectively. Nevertheless, no two males shared all song types in their repertoires and all males shared some song types. We found a significant negative correlation between the level of song types shared between two males and their proximity (Mantel r = -0.49, P>0.05).

In all, we identified a total of 78 song types in the song repertoires of eight males. Out of 78, four song types (5%) were common and were delivered by all the males, while 33 (42%) song types were each delivered by one male only (Figure 3). The patterns of song type sharing between male pied bush chats are summarized in Table 1.

DISCUSSION

Our results indicate that territorial neighbouring male pied bush chats shared songs and that neighbours shared significantly more song types than did non-neighbours. Most studies have suggested a relationship between sedentary behaviour and higher levels of song sharing between territorial neighbours (McGregor & Krebs, 1989; Beecher et al., 1994; Rogers, 2004) and our study also supports this correlation. In this study, males did not migrate and were always found in their respective territories throughout the year. Such sedentary habits might allow birds to breed in the same area where they were born and learned their songs (Beecher et al., 1994). Alternatively, males could exhibit song

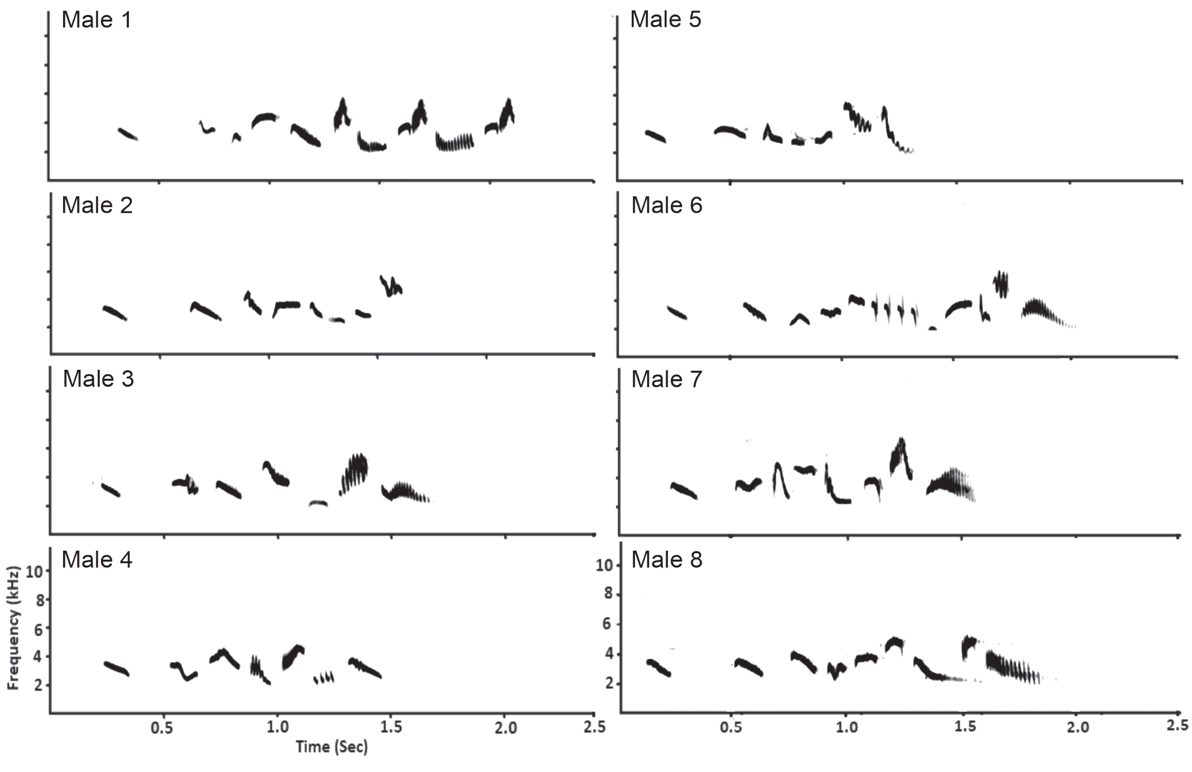

Fig. 3. – Spectrograms of unshared song types from the song repertoires of eight males.

TABLE 1

Patterns of song type sharing in male pied bush chats (N= 8).

No. of song types (n= 78)	No. of males that shared particular song type		Percentage
33	Unshared (i.e. sung by 1 male only)		42.3
20	2		25.6
10	3		12.8
8	4		10.3
1	5		1.3
1	6		1.3
1	7		1.3
4	8		5.1
		Total	100

sharing either by adjusting their song repertoires to the neighbouring males or by settling next to males with similar song repertoires (DEWOLFE et al., 1989; PAYNE & PAYNE, 1993).

Song sharing among neighbouring male songbirds seems to result from at least three non-mutually exclusive processes based on the time of song memorization and song production (NELSON, 1992). The first model suggests that young birds learn their song repertoires from their neighbours or father before dispersal and then settle close to them (MARLER & TAMURA, 1962; CUNNINGHAM & BAKER,1983; GRANT & GRANT,1996). The second model suggests that song acquisition occurs after natal dispersal when males establish a territory for the first time (KROODSMA, 1974; PAYNE & PAYNE, 1993). A third model, termed action-based learning (MARLER, 1990), suggests that males produce a variety of songs in their plastic song stage and then select a subset of song(s) for retention in their repertoires based on social interactions with their neighbours (DEWOLFE et al., 1989). However, we do not have any data on song learning by young pied bush chat. Therefore, based on the present study, it is difficult to comment on the evolution of song sharing in the pied bush chat.

The decline of song sharing with distance in the pied bush chat seems to be consistent with the song learning model of BEECHER et al., (1994, 1996) developed for the western population of the song sparrow. According to this model, young song sparrows memorize songs about two to three months following nutritional independence. During this time, they visit the territories of several adult males to establish their own territory and learn songs from these adult males. The young birds either succeed in occupying a territory near their tutors and exhibit a high level of song sharing with them or they establish their territories farther away from their tutors and exhibit less or no song sharing with them. However, the song learning behaviour in the pied bush chat has not been studied so far and therefore further studies seem necessary to understand the effect of song learning on the song sharing behaviour of this species.

The present study indicates that song sharing is higher between close neighbours than between distant birds. Similarly, a study of syllable sharing in the house finch (*Carpodacus mexicanus*) has revealed a negative correlation between the extent of syllable sharing and distance (BITTERBAUM & BAPTISTA, 1979). Similar relationships have been suggested for Bewick's wren (*Thryomanes bewickii*, KROODSMA, 1974), American redstart (*Setophaga ruticilla*, LEMON et al., 1994), song sparrow (WILSON et al., 2000), and chowchilla (*Orthonyx spaldingii*, KOETZ et al., 2007). In sedentary species, especially those occupying permanent territories such as the pied bush chat, a distance of about 2 km between neighbouring groups might create acoustic isolation, with males possibly out of earshot from each other. Therefore, in this study, distant males could probably not hear each other and did not show higher levels of song sharing. A similar effect of isolation by distance has been suggested for the great tit (*Parus major*) in which males in closer forest fragments share more song types and the highest levels of sharing are observed among males breeding in the same forest fragment (RIVERA-GUTIERREZ et al., 2010).

Evidence from a variety of species suggests that song sharing, in the form of song-type matching or repertoire-matching, allows males to interact vocally in ways that are impossible without sharing (HUGHES et al., 2007; BEECHER et al., 2000; SEARCY & BEECHER, 2009). Song-type matching may result in escalated aggressive contexts or may be followed by close aggressive approach (NIELSEN & VEHRENCAMP, 1995) while repertoire-matching may direct mild threats to a specific neighbour without escalating the encounter (BEECHER et al., 1996). However, it has been suggested that song matching does not necessarily always evolve through aggressive encounters. It may also help females to compare males and other males to assess the two singers (LOGUE & FORSTMEIER, 2008). Overall, song sharing has the potential to indicate the level of aggression, motivation, experience, abilities, or intent of the territorial singers (SEARCY & BEECHER, 2009; NAGUIB & MENNILL, 2010; PRICE & YUAN, 2011). Although in this study, we did not investigate the type of song sharing (song-type match vs. repertoire match), yet we frequently observed neighbouring males coming to the boundary of their territories, facing each other and performing counter-singing. Such counter-singing between neighbouring males remained common throughout the breeding season suggesting an important role in territorial interactions between neighbouring males. It seems that song sharing in the pied bush chat might help males to mediate social relationship among them to proclaim an established territory. Our previous observations have also suggested that male pied bush chats sing primarily for close-range communication among neighbouring males to defend their territory (SETHI et al., 2011b).

In conclusion, this study reports the existence of song sharing in the Pied Bush Chat, where neighbouring males share significantly more song types than do non-neighbouring males. We report a decline in the proportion of song repertoires shared with increasing distance between males. Observations also indicate that song sharing in the pied bush chat possibly helps males mediating social relationships via improved acquisition and maintenance of territories. Further experimental studies would be necessary to ascertain the type of song sharing (repertoire or song-type matching) that occurs between neighbouring males along with understanding the song learning behaviour and adaptive significance of song sharing in the pied bush chat.

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Reproductive activities of two zooplanktivorous clupeid fish in relation to the seasonal abundance of copepod prey in the northern end of Lake Tanganyika

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ABSTRACT. Reproductive activities of two commercially exploited clupeid fishes (*Stolothrissa tanganicae* and *Limnothrissa miodon*) in the northern end of Lake Tanganyika (Bujumbura sub-basin) were investigated during two different years (2004-2005 and 2007-2008). We hypothesized that the timing of largest reproductive investment in these pelagic species coincides with the onset of the rainy season and the highest abundance of copepod prey. For *S. tanganicae*, the gonadosomatic index (GSI) was significantly higher prior to the onset of the rainy season, and this was observed in both years. For *L. miodon*, however, this pattern was overall weaker and only statistically significant in 2004-2005. In both species, spawning activities did not seem to be adjusted to provide optimal feeding conditions for the larvae. Instead, investment in reproduction seemed to be related to the availability of copepods in the preceding months. We conclude that the timing of reproductive activities in these clupeids is species-specific, and might be subject to strong year-to-year variation.

KEY-WORDS: Clupeidae, copepod, fisheries, gonadosomatic index, reproductive activities

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INTRODUCTION

Lake Tanganyika is the oldest of the East African Great Lakes (COULTER, 1991) and is the second largest and deepest freshwater body in the world. Up to now, around 1200 species have been identified in this lake, classifying it at second position in biodiversity (COHEN et al., 1993). Among the main groups, the fishes show a high degree of biodiversity (VAN STEENBERGE et al., 2011). The last whole-lake overview indicated 250 cichlid species (95 % endemics) and 75 non-cichlid species (59 % endemics) (SNOEKS, 2000).

Stolothrissa tanganicae REGAN, 1917, and *Limnothrissa miodon* (BOULENGER, 1906) (Phylum Chordata, Class Osteichthyes) are commercially-exploited clupeid fishes that provide a vital source of livelihood and food supply to more than 10 million people living in the surroundings of Lake Tanganyika (MÖLSÄ et al., 1999). Together they represent 60% (MÖLSÄ et al., 1999) to 90% (MULIMBWA, 2006) of the total pelagic commercial fish catches from Lake Tanganyika. Unfortunately, recent statistics show that the amount of catch is decreasing, at least in the northern part of the lake (MULIMBWA, 2006; LTA SECRETARIAT, 2012). Some authors have related decreases of catch to climate change (PLISNIER, 1997; O’REILLY et al. 2003), but so far overfishing is likely to be the major reason because of the excessive fishing pressure that has doubled since the mid-1990s (MULIMBWA, 2006; SARVALA et al., 2006a, 2006b; VAN DER KNAAP et al., 2014). This applies particularly to the northern part of the lake, which has the highest fishing effort per km of shoreline due to high densities of lift nets and traditional fishing units (MÖLSÄ et al., 1999; VAN DER KNAAP et al., 2014).

The biology of *S. tanganicae* and *L. miodon* has been the subject of several studies supporting fisheries management. These investigations have documented life history (MARLIER, 1957), life cycle and length at sexual maturity (MANNINI et al., 1996), population dynamics (SHIRAKIHARA et al., 1992), reproduction and recruitment (MULIMBWA & SHIRAKIHARA, 1994), and spatio-temporal distribution (PLISNIER et al., 2009). Several studies also investigated the feeding biology of both species (POLL, 1953; CHÈNÉ, 1975; MANNINI et al., 1996; LENSU, 1998; ISUMBISHO et al., 2004), as well as the ecology and distribution of zooplankton as their most important prey category (NARITA et al., 1985; MULIMBWA, 1988 and 1991; COENEN, 1995; BWEBWA, 1996; KURKI et al., 1999; SARVALA et al., 1999; VUORINEN et al., 1999). Larvae of *S. tanganicae* have been reported to feed on phytoplankton (CHÈNÉ, 1975), but later studies suggest that their main food source is copepod nauplii (Lake Tanganyika Research Project, unpublished [1998]). Adults mainly feed on copepods, particularly *Tropodiaptomus simplex* (SARS, 1909) (CHÈNÉ, 1975; MGANA et al., 2014); bigger adults also take pelagic shrimps (LENSU 1998; see also COULTER, 1991). In Lake Kivu, larvae of *L. miodon* (10-35 mm) feed preferentially on copepods (nauplii), but also on adult cladocerans, chironomid larvae, Ephemeroptera larvae, vegetal debris, diatoms, Chlorophyceae filaments and organic rubbish (DE IONGH et al., 1983). Juveniles of *L. miodon* (30-70 mm) from lake Kivu feed preferentially on copepods, but also on chrysophytes, *Microcystis*, Rotifera, insect larvae, and nauplii (DE IONGH et al., 1983). In Lake Kivu, adults of *L. miodon* (85 – 110 mm) feed preferentially on chironomids, juveniles of *L. miodon*, copepods, *Microcystis*, land insects, and Trichoptera (DE IONGH et al., 1983). In Lake Tanganyika, adult *L. miodon* also feed on larvae and juveniles of *S. tanganicae*, as well as on pelagic shrimps (COULTER, 1991; MANNINI et al., 1996; LENSU, 1998); *L. miodon* is hence more omnivorous than *S. tanganicae*, in part due to its long littoral phase during which it is using more varied food items.

In this study, we aim to further deepen the knowledge on the biology of these clupeid fishes by describing the timing of their reproductive activities. For *S. tanganicae*, there seems little agreement on the precise timing of reproduction. Sexually mature fish occur throughout the year (ELLIS, 1971) and individuals may spawn several times in a year (COULTER, 1961). Spawning peaks as indicated by variation in the relative number of ripe gonads were apparent in November-December and in April-July in Zambia (ELLIS, 1971), i.e. respectively in the beginning and at the end of the rainy season and well into the dry season. On the other hand, fry abundance in Zambia suggested a major annual spawning at the end of or right after the dry season, i.e. in August-December (COULTER, 1970), and length frequencies of older fish indicated a September peak (PEARCE, 1985). In Tanzania (Kigoma) there appears to be a peak in January-April (CHAPMAN & VAN WELL, 1978), while in the north in Burundi the main spawning is slightly later, in February-May (ROEST, 1977). The seasonal length-frequency distributions suggest usually four annual spawning peaks of varying strength in both Burundi and Uvira waters (MÖLSÄ et al., 2002; MULIMBWA et al., 2014). For *L. miodon*, it seems generally agreed that the main spawning takes place during the rainy season between November and May (MATTHES, 1967; ELLIS, 1971; PEARCE, 1985; MULIMBWA & SHIRAKIHARA, 1994), although fry occur throughout the year, and a peak of spawning was recorded from August to October in the north end of the lake (ARO & MANNINI, 1995).

What factors influence the timing of reproductive investment in both species? A number of studies demonstrated that the abundance of clupeid fishes is influenced by climatological conditions and associated limnological parameters (e.g. PLISNIER et al., 2009). It is therefore likely that climatological and limnological fluctuations also trigger reproductive activities. Recently, Mulimbwa et al. (2014) investigated the relationship between seasonal changes in the pelagic catch of both clupeid species and the abundance of copepod prey. It was observed that peaks in the abundance of copepods from the onset of the rainy season onwards (October-April) were correlated with the appearance of strong cohorts of both *S. tanganicae* and *L. miodon*. However, various strong cohorts in both species clearly did not match with peaks in the abundance of copepods. Small *S. tanganicae* (2-4 months old) were observed almost year round except in February, May and November-December. The smallest *L. miodon* were around in July-September, November, and March-April. It therefore remains unclear whether or not *S. tanganicae* and *L. miodon* synchronize their reproductive investment with the onset of the rainy season and the highest abundance of copepod prey (i.e. from October-November onwards), which would be most favourable for their offspring. In addition, the reproductive effort of the clupeids might be regulated by the availability of zooplankton food for the

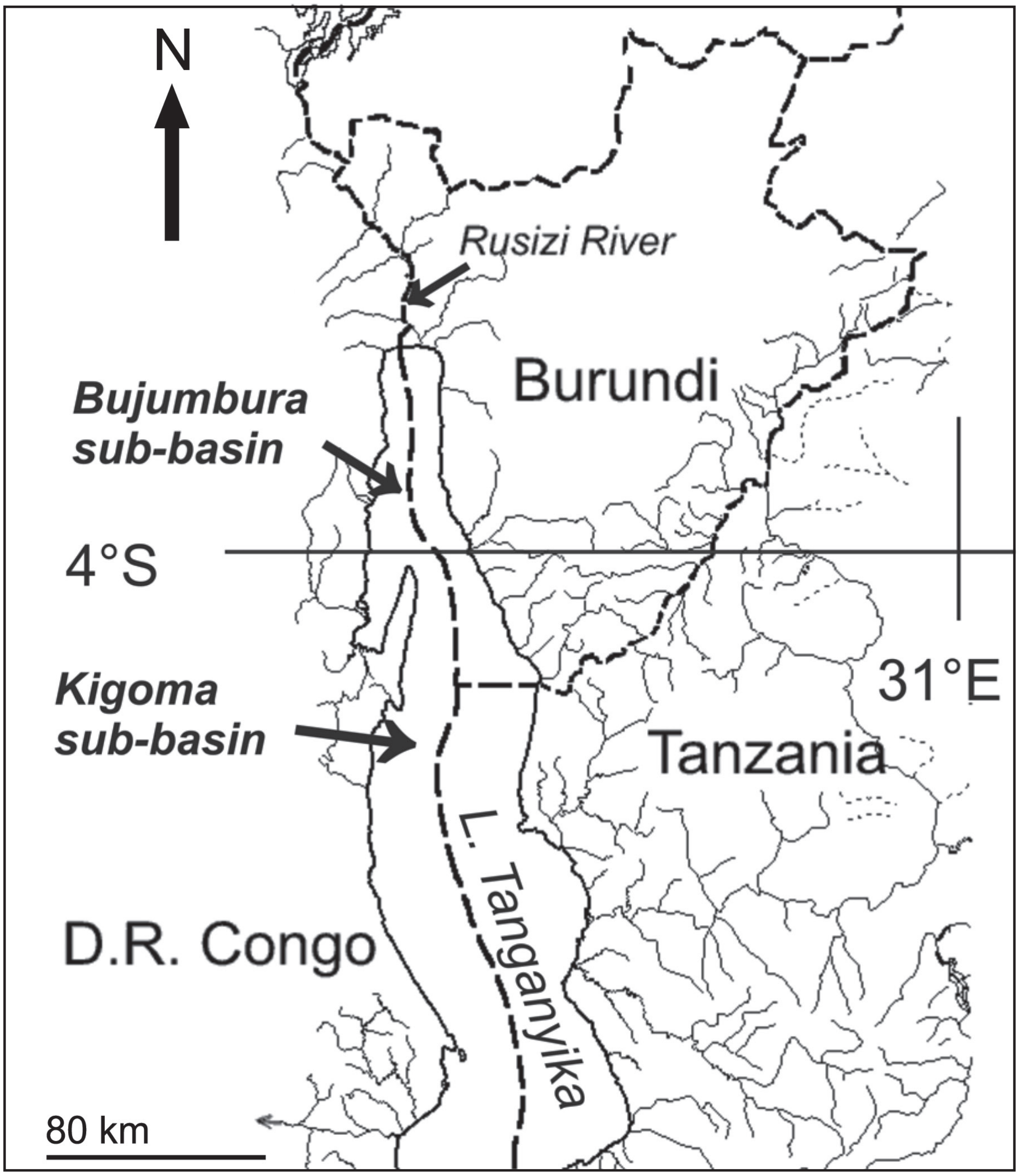

Fig. 1. – Map of the north part of Lake Tanganyika, including the Bujumbura sub-basin.

adults before the spawning period. To test these hypotheses, we document here the reproductive activities of *S. tanganicae* and *L. miodon* relative to the seasonality of zooplankton in the northern end of Lake Tanganyika (Bujumbura sub-basin).

MATERIAL AND METHODS

Data collection

The study was performed in the Bujumbura sub-basin at the north end of Lake Tanganyika (Fig. 1; 03° 28' S and 29° 17' E). Fish samples were obtained from local fisheries from November 2004 to October 2005 and from March 2007 to February 2008. Rainfall data for these periods were collected at the Hydrobiological Research Center (C. R. H.) in Uvira and summed up on a monthly basis. Copepod data were obtained from February 2007 to January 2008, i.e. only for the second period.

Sampling of copepods was carried out twice a month in the pelagic area 7 km from the shore, as described in MULIMBWA et al. (2014). The lake is more than 100 m deep here. Samples were concentrated to a volume of 40 to 60 ml, from which three 1 ml subsamples were taken after thorough mixing. Copepods in these subsamples were identified and counted under a microscope. The copepod taxa and various developmental stages (nauplii, copepodids, males, females, and females with eggs) were identified according to ALEKSEEV (2002). Calanoid copepods were represented by only one species, *Tropodiaptomus simplex*. The cyclopoid copepods were grouped into small and large size classes, the small one referring to all copepodid and adult stages of *Tropocyclops tenellus* (SARS, 1909) and the large one to those of *Mesocyclops aequatorialis* KIEFER, 1929. Naupliar stages of cyclopoid copepods were counted as one group and mainly represent *M. aequatorialis*.

Fish samples were taken twice a week from artisanal lift net fishing units at the time of landing. In the artisanal fishery in the northwestern part of Lake Tanganyika, a fishing unit is composed of two or three wooden boats of 8.0 m x 1.4 m, lift net size with 16 m × 16 m mouth opening and 20 m depth. The mesh size of the nets is 8 – 10 mm in the upper four fifths and 6 – 7 mm in the lower one fifth. Each fishing unit uses 17 to 20 lamps and is operated by eight fishermen. With regard to *S. tanganicae* and *L. miodon* about a handful of fresh fish was taken as a sample from four fishing units. A minimum of 70 mm total length (TL) was considered, which corresponds to the minimal size at which these fishes are able to reproduce (ELLIS, 1971). In 2004-2005, 136 male *S. tanganicae*, 310 female *S. tanganicae*, 213 male *L. miodon*, and 243 female *L. miodon* were obtained. In 2007-2008, 128 male *S. tanganicae*, 275 female *S. tanganicae*, 192 male *L. miodon*, and 264 female *L. miodon* were included. Fish were measured and weighed, and their gonads were dissected and weighed to the nearest 0.01g. For 2007-2008, the gonads were inspected under a dissecting microscope and classified as mature (stage IV) or immature (stage I, II or III) according to a scale of gonad maturity based on gonad size and egg development (DE KIMPE, 1964; MICHA, 1973). Males were considered mature when testes were white and sperm ducts were filled with sperm; females were considered mature when ovaries were light yellow to orange and oviducts were filled with ovules. Individuals that had already reproduced (stage V) were not encountered.

Between August 2009 and July 2010, additional sampling was performed by an experimental fishing unit, targeting the larvae of *L. miodon*, which hatch on the sand in the shallow water, close to the shoreline (MATTHES, 1967; COULTER, 1970; PEARCE, 1985). Four different beaches (Kalundu Congo SEP (mouth of Rusozi River), Kamongola, mouth of Kalimabenge River, and CRH Guest house) were monitored for this purpose, from 6h30 to 7h30 in the morning. The larvae were sampled by two persons by trawling a mosquito net (width: 2 m; height: 1 m) over a distance of 50 m, keeping the lower part of the net close to the bottom and the upper part at the surface. A third person chased the fish larvae

TABLE 1

Average individual biomass of three copepod species (µg carbon) based on direct determinations with a carbon analyser (adapted from SARVALA et al., 1999).

Species	*Tropodiaptomus simplex*	*Mesocyclops aequatorialis*	*Tropocyclops tenellus*
Males	2.01	1	0.16
Females	2.95	2.281	0.183
Females with eggs	3.3	2.315	0.186
Copepodid	0.717	0.487	0.132
Nauplius	0.153	0.098	0.05

towards a central pocket. The total weight and the modal length of the larvae in samples were determined, and the weekly mean values across the four beaches were calculated.

Data analysis

Copepod counts were extrapolated to individuals per cubic meter, and two-week copepod abundances were calculated as the total number of copepod individuals per cubic meter. Monthly values were then calculated as the average of the two-week data. Individual carbon mass values, based on direct determinations of Tanganyika zooplankton, were derived from the literature (Table 1; SARVALA et al., 1999). Total sampled copepod biomass per month was calculated by multiplying the monthly abundance of each species and stage by the appropriate average individual mass.

Reproductive activities of the two clupeids were studied using the gonadosomatic index (GSI) of individuals larger than 70 mm TL. The GSI was defined as the percentage of gonad weight of total body weight. For 2007-2008, the monthly percentage of mature gonads was calculated as an additional measure of reproductive activity.

Student's t tests were used to test the differences in rainfall, and copepod abundances and biomass between the rainy season (October to April) and the dry season (May to September). ANOVA, followed by post hoc Tukey tests, was used to test the fluctuations between months for the GSI. As the variation in GSI between months might be influenced by cohort structure and size differences, month effects on GSI were also tested with an ANCOVA including total length as a covariate. For 2007-2008, a chi-square test was used to evaluate the significance of month-to-month fluctuations in the percentage of mature gonads.

Pearson correlations and linear regressions were calculated with the program package PAST (HAMMER et al., 2001) to examine the relationships between the monthly changes in clupeid GSI and variations in zooplankton food available. To check whether clupeid reproductive effort might simply reflect the availability of food, GSI was compared with the biomass of copepod prey during the concurrent and 1-3 previous months, both separately and as averaged combinations of 2-4 months. Further, to check whether the timing of clupeid reproduction was adjusted to optimise food availability for the larval and young fish, GSI was compared with the biomass of copepod food in the following month.

RESULTS

Rainfall and copepods

Total rainfall in 2004-2005 (1335 mm) was considerably higher than in 2007-2008 (814 mm). In 2004-2005, the monthly rainfall was high from December until May, and low from June until

October (Fig. 2A). In 2007-2008, a similar pattern with slightly different timing was observed with moderate to strong rainfall from February until May, moderate to weak rainfall from June to September, and again moderate to strong rainfall from October until January. Similar fluctuations were observed for total copepod biomass in 2007-2008 with high values in February, April

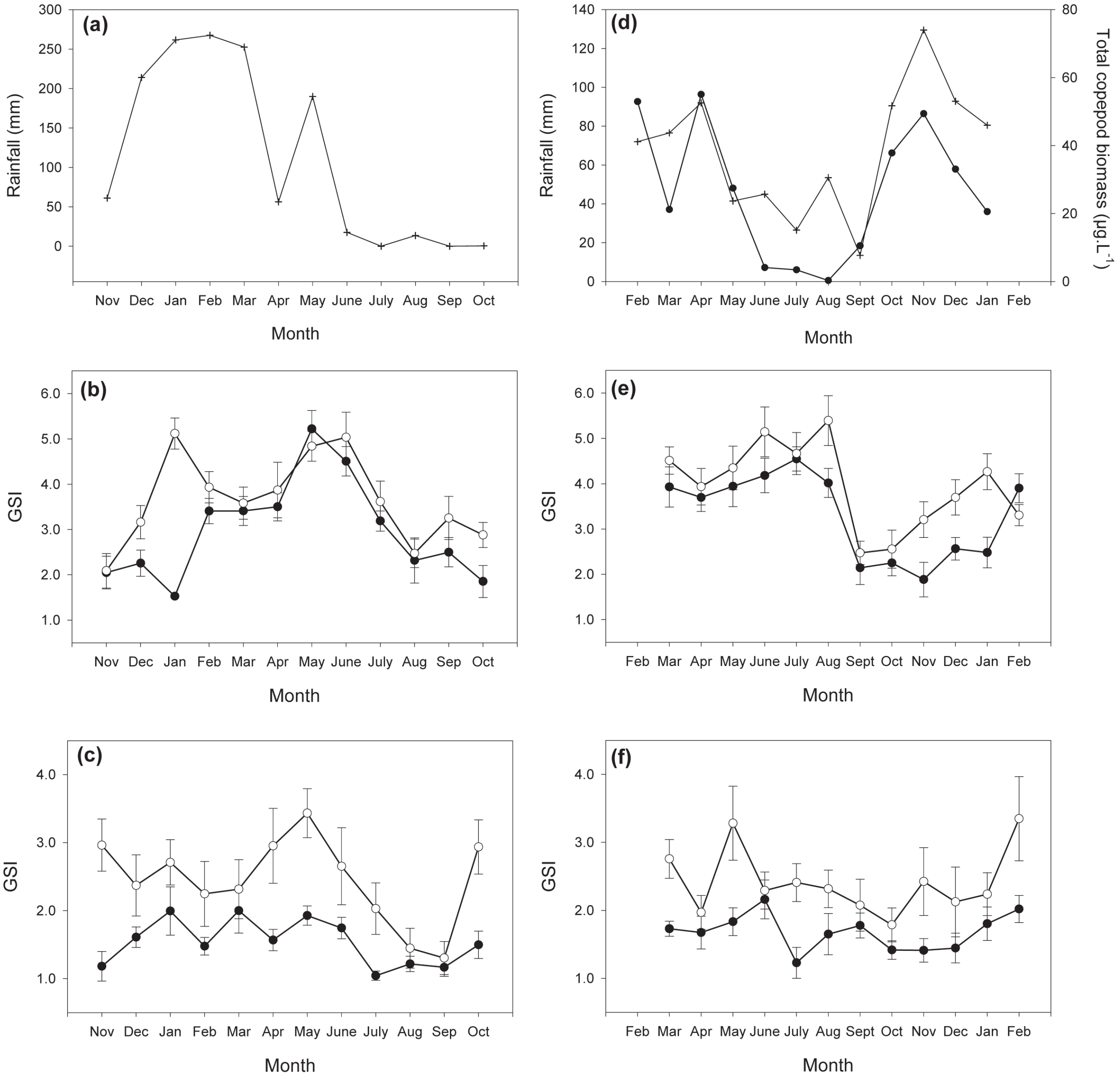

Fig. 2. – Monthly rainfall, total copepod biomass, and reproductive investment of two clupeid fishes in the northern end of Lake Tanganyika, from November 2004 until October 2005 (left) and from February 2007 until February 2008 (right). A) Average monthly rainfall (mm) in 2004-2005. B) Gonadosomatic index in males (black dots) and females (circles) of *Stolothrissa tanganicae* in 2004-2005. C) Gonadosomatic index in males (black dots) and females (circles) of *Limnothrissa miodon* in 2004-2005. D) Average monthly rainfall (left axis in mm; cross marks) and total copepod biomass (right axis in µg carbon L^{-1}; black dots) in 2007-2008 (data from MULIMBWA et al., 2014). E) Gonadosomatic index in males (black dots) and females (circles) of *Stolothrissa tanganicae* in 2007-2008. F) Gonadosomatic index in males (black dots) and females (circles) of *Limnothrissa miodon* in 2007-2008. Vertical bars represent standard errors.

TABLE 2

Differences between the rainy (October to April) and dry season (May to September) of 2007-2008 in rainfall and the abundances and biomasses of the main zooplankton categories and the total copepods (SE: standard error; C+A: copepodids and adults; Student's t-test assuming unequal variances; significant P-values in bold; $n_{rainy}=7$; $n_{dry}=5$).

Variable	Mean rainy season (SE)	Mean dry season (SE)	t	P
Rainfall (mm month^{-1})	90.5 (7.2)	36.0 (7.1)	5.39	**0.0003**
Copepod abundance (ind. L^{-1})				
Tropodiaptomus simplex C+A	6.7 (1.7)	1.3 (0.5)	3	**0.02**
Mesocyclops aequatorialis C+A	21.3 (8.3)	6.2 (4.1)	1.62	0.14
Tropocyclops tenellus C+A	3.5 (1.2)	1.2 (0.6)	1.71	0.13
Copepoda nauplii	97.9 (24.7)	22.1 (17.7)	2.49	**0.03**
Total Copepoda	129.3 (24.5)	30.7 (22.5)	2.97	**0.01**
Copepod biomass (µg carbon L^{-1})				
Tropodiaptomus simplex C+A	11.7 (3.6)	2.2 (0.8)	2.58	**0.04**
Mesocyclops aequatorialis C+A	16.1 (4.6)	4.5 (2.4)	2.26	**0.05**
Tropocyclops tenellus C+A	0.5 (0.2)	0.2 (0.1)	1.79	0.11
Copepoda nauplii	9.9 (2.5)	2.3 (1.8)	2.51	**0.03**
Total Copepoda	38.2 (5.4)	9.2 (4.9)	4.01	**0.002**

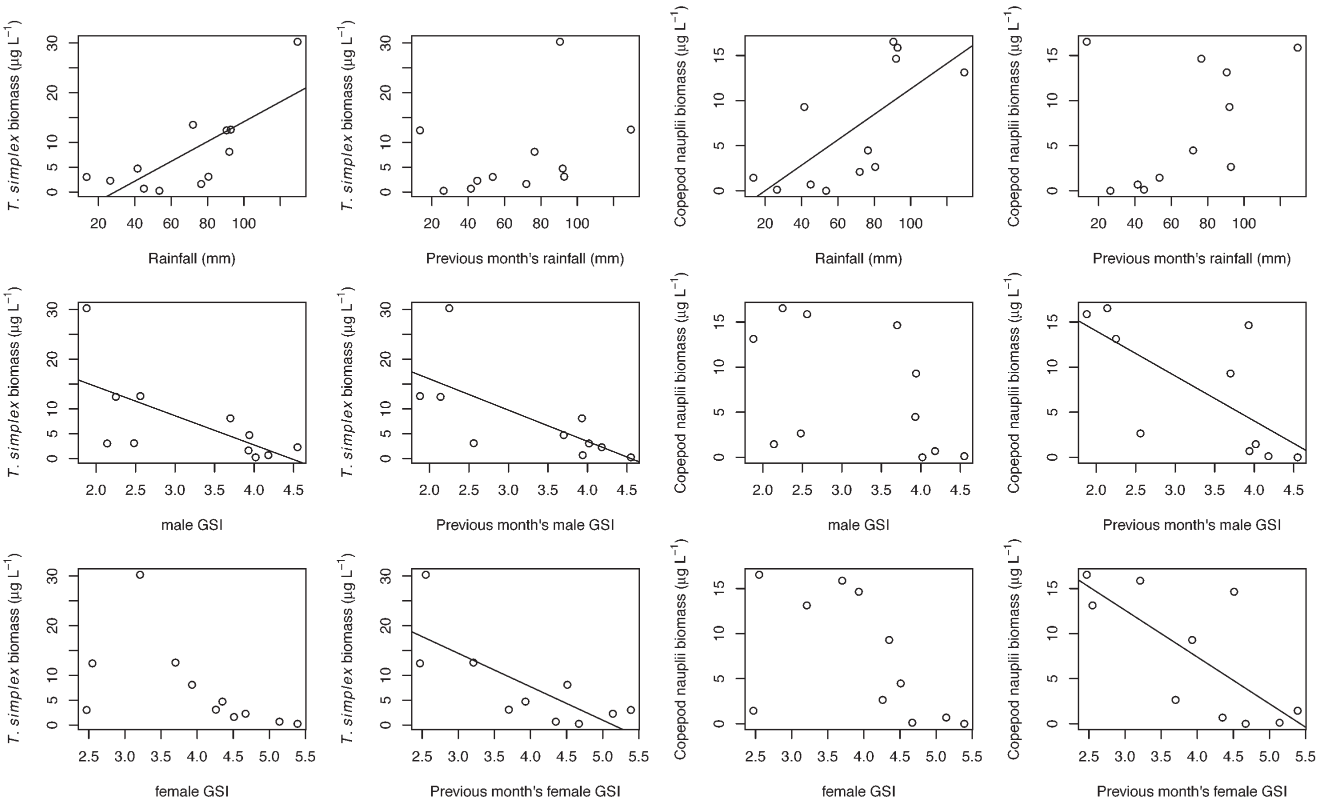

Fig. 3. – Rainfall and gonadosomatic index (GSI) in male and female *Stolothrissa tanganicae* in the concurrent and preceding month versus *T. simplex* copepodid and adult biomass and copepod nauplii biomass, from March 2007 until February 2008. Relationships marked with a regression line are significant.

TABLE 3

Mean GSI in the rainy season (October to April) and the dry season (May to September) and ANOVA/ANCOVA between months for males (m) and females (f) of two clupeid fishes. P-values in bold indicate significant differences between months before (ANOVA) and after (ANCOVA) controlling for total length. Note that the GSI was consistently higher in the dry season than in the rainy season for *S. tanganicae*, but not for *L. miodon*. See Figure 2 for month-to-month fluctuations in GSI. SE: standard error.

		Averages by season		**ANOVA between months**		**ANCOVA between months**	
Year	Species (sex)	Mean rainy season (SE)	Mean dry season (SE)	F	p	F	p
2004-2005	*S. tanganicae* (m)	2.77 (0.15)	3.79 (0.20)	$F_{11,124}$=8.95	**< 0.0001**	$F_{11,123}$=9.89	**< 0.0001**
	S. tanganicae (f)	3.51 (0.15)	4.01 (0.22)	$F_{11,298}$=6.12	**< 0.0001**	$F_{11,297}$=5.95	**< 0.0001**
	L. miodon (m)	1.59 (0.08)	1.39 (0.07)	$F_{11,200}$=3.58	**0.0001**	$F_{11,199}$=3.63	**0.0001**
	L. miodon (f)	2.68 (0.16)	2.28 (0.19)	$F_{11,231}$=2.45	**0.0066**	$F_{11,230}$=2.44	**0.0067**
2007-2008	*S. tanganicae* (m)	2.91 (0.15)	3.83 (0.20)	$F_{11,112}$=6.36	**< 0.0001**	$F_{11,111}$=7.15	**< 0.0001**
	S. tanganicae (f)	3.65 (0.14)	4.26 (0.23)	$F_{11,263}$=5.65	**< 0.0001**	$F_{11,260}$=4.59	**< 0.0001**
	L. miodon (m)	1.64 (0.07)	1.79 (0.11)	$F_{11,180}$=1.25	0.2552	$F_{11,177}$=0.99	0.4607
	L. miodon (f)	2.36 (0.14)	2.34 (0.14)	$F_{11,252}$=1.38	0.1838	$F_{11,251}$=1.55	0.1149

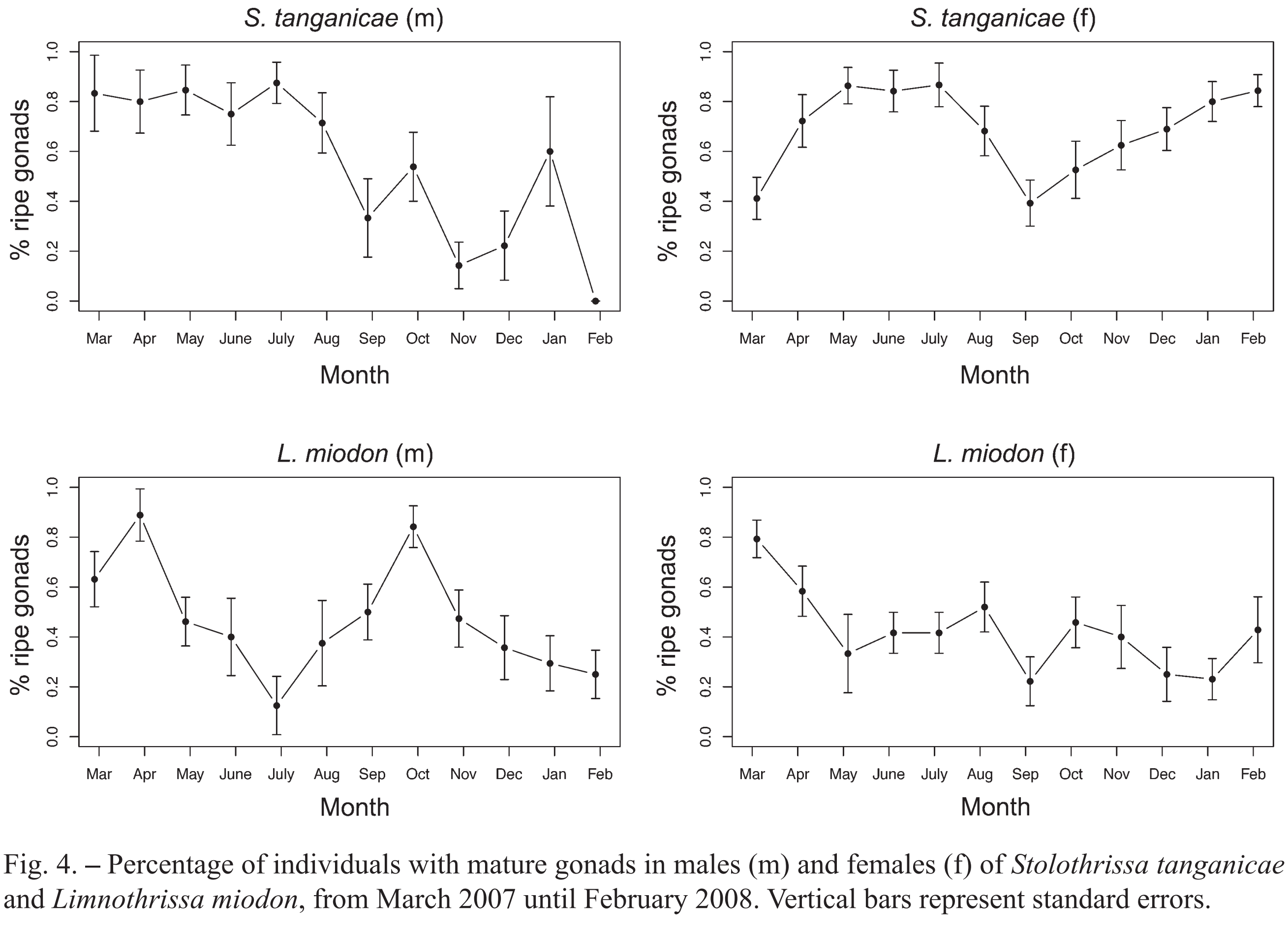

Fig. 4. – Percentage of individuals with mature gonads in males (m) and females (f) of *Stolothrissa tanganicae* and *Limnothrissa miodon*, from March 2007 until February 2008. Vertical bars represent standard errors.

and November, and low values from June until September (Fig. 2D). Differences between the rainy and dry seasons were statistically significant for rainfall, *M. aequatorialis* copepodid and adult biomass, and the abundance and biomass of *T. simplex* copepodids and adults, copepod nauplii and total copepods (Table 2). Accordingly, copepod biomass was positively correlated with the rainfall of the current month (rainfall vs. *T. simplex* copepodid and adult biomass: r = 0.76, p = 0.0039, df = 10, Fig. 3; rainfall vs. copepod nauplii biomass: r = 0.70, p = 0.0118, df = 10, Fig. 3; rainfall vs. total copepod biomass: r = 0.74, p = 0.0060, df= 10). In contrast, none of the copepod biomass categories was significantly correlated with the previous month's rainfall (Fig. 3).

Clupeid reproductive investment

Stolothrissa tanganicae – Males ranged between 72 mm and 103 mm TL (average: 87 mm), while females were 71-110 mm TL (average: 90.8 mm). In 2004-2005, male GSI fluctuated significantly between months, with high values in May and June and low values from November to January and August to October (Fig. 2B; Table 3). In 2007-2008, a broader peak was observed from March to August, with a significant drop towards September (Fig. 2E; Table 3). Female GSI also fluctuated significantly between months (Figs 2B, E, Table 3). In 2004-2005, values were generally high from January to July, with a peak in January and a second peak from May to June (Fig. 2B). In 2007-2008, female GSI was high from March to August with a steep drop to low values in September-October; a new peak was evident in January (Fig. 2E). In both sexes and both years, the differences in GSI between months remained significant when GSI was controlled for total length (ANCOVA; Table 3), indicating that GSI fluctuations between months were not or not exclusively due to shifts in cohort structure, but to differential reproductive investment. GSI was also consistently higher in the dry season than

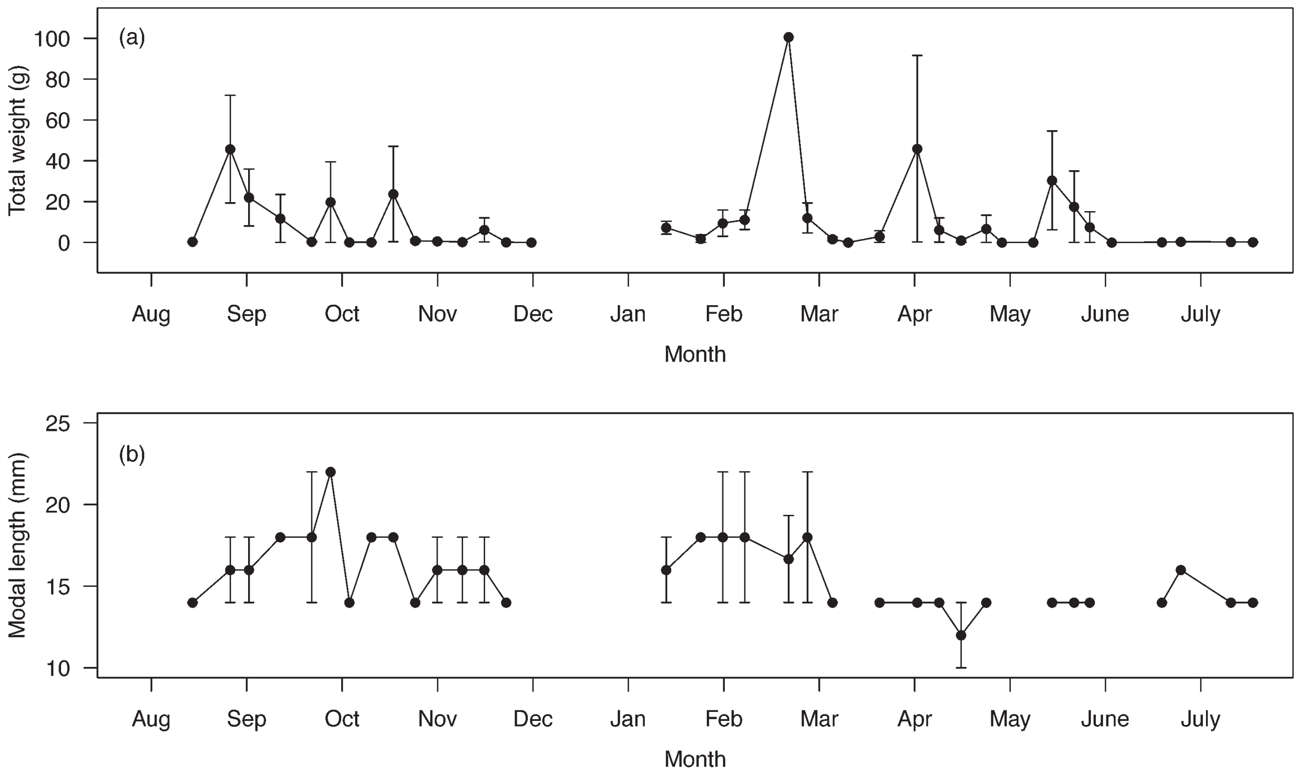

Fig. 5. – Seasonal changes in the weight (g) of the total catch and the modal length (mm) of *Limnothrissa miodon* larvae in the littoral samples between August 2009 and July 2010. Values represent the average of the captures at four different beaches. Vertical bars represent standard errors.

TABLE 4

Pearson correlations of clupeid GSI with the rainfall of the corresponding and the preceding month (combined data for 2004-2005 and 2007-2008). Stan = *S. tanganicae*, Lmio = *L. miodon*.

	Stan male	Stan female	Lmio male	Lmio female	df
Rainfall, same month	-0.061	0.167	0.408	0.198	21
Rainfall, preceding month	0.119	0.306	0.375	0.222	22

in the rainy season (Table 3). In 2007-2008, the percentage of males and females with mature gonads steeply decreased towards the end of the dry season, suggesting a major spawning peak in August-September (Fig. 4). These fluctuations were significant (males: $\chi^2 = 43.2$, $p < 0.0001$, df = 11; females: $\chi^2 = 37.6$, $p < 0.0001$, df = 11).

Limnothrissa miodon - Males ranged between 74 mm and 129 mm TL (average: 94.5 mm), while females were 75-136.4 mm TL (average: 100.8 mm). In 2004-2005, male GSI was high from January to June, and dropped significantly in July (Fig. 2C, Table 3). Female GSI showed a significant peak in November, May and October, and the lowest values in August-September

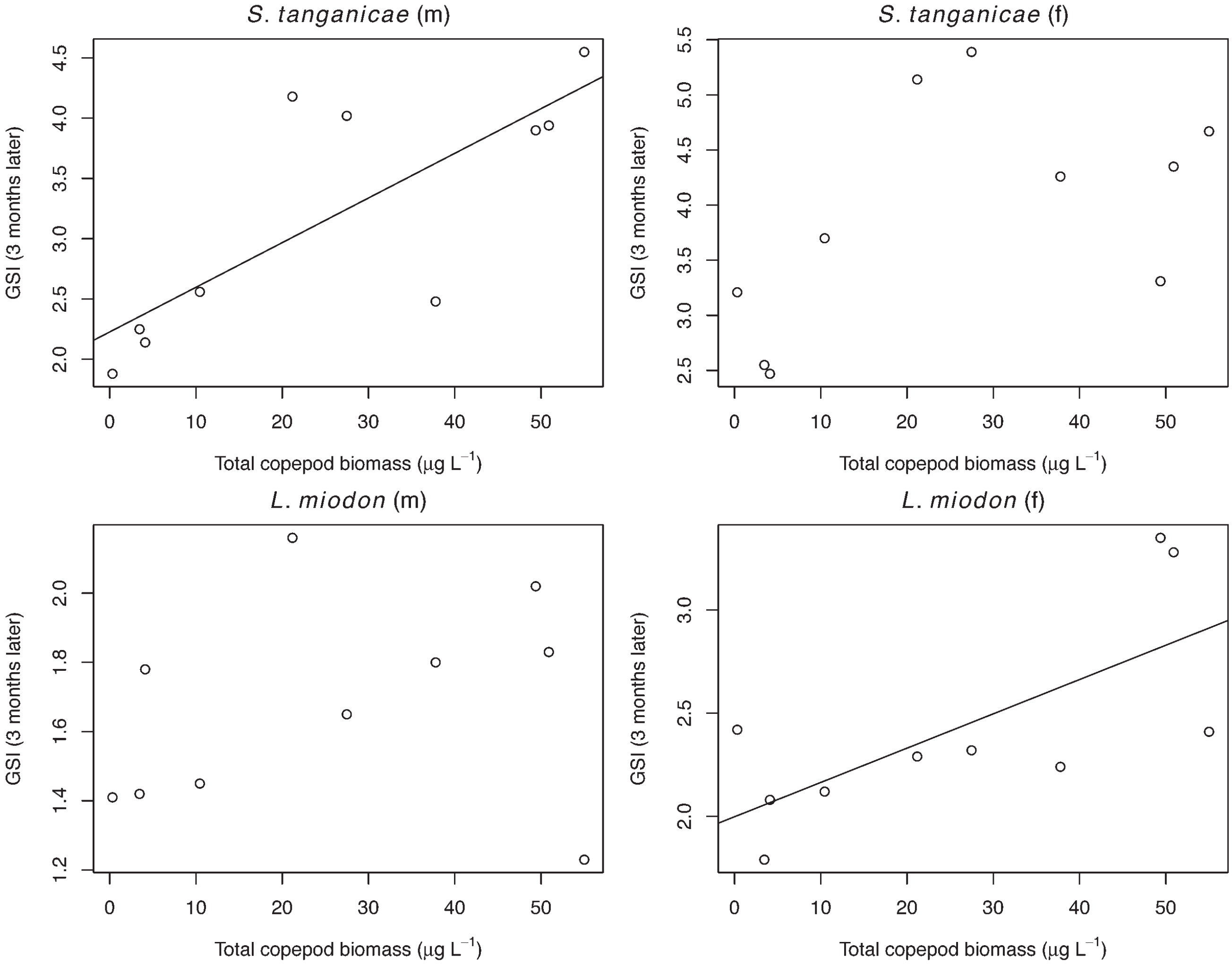

Fig. 6. – Total copepod biomass versus the average monthly gonadosomatic index (GSI) three months later in two clupeid fishes, from March 2007 until February 2008. Relationships marked with a regression line are significant.

(Fig. 2C, Table 3). In 2007-2008, male GSI showed a subtle peak in June, while for females reproductive activity peaked in March, May and February (Fig. 2F). However, none of the differences in 2007-2008 were statistically significant. None of these results changed when GSI was controlled for total length (ANCOVA; Table 3). In contrast to *S. tanganicae*, there was no consistent difference in GSI between the rainy season and the dry season across years and sexes (Table 3). In 2007-2008, the percentage of males with mature gonads peaked in April and October, while the highest percentage of females with mature gonads was observed in March (Fig. 4). These fluctuations were significant (males: $\chi^2 = 29.9$, p = 0.0016, df = 11; females: $\chi^2 = 28.45$, p = 0.0028, df = 11).

Weekly captures by an experimental fishing unit at four different beaches revealed that *L. miodon* larvae predominated in the rainy season (January to May), but were also abundant at the end of the dry season (August to September) (Fig. 5). The modal length of the larvae was, most of the time, less than 19 mm, suggesting that they were less than 1 month old (deduced from length growth curves in Mulimbwa et al., 2014).

Reproductive investment vs. rainfall and copepods

GSI versus rainfall - Clupeid GSI did not show significant correlation with the rainfall of the concurrent or the previous month (combined data for the years 2004-2005 and 2007-2008; Table 4).

GSI versus copepods in the corresponding and following month - In both clupeids, the monthly GSI showed no relationship with copepod biomass of the corresponding month (total copepod biomass or that of the separate zooplankton groups), except for a significant negative correlation between male *S. tanganicae* GSI and *T. simplex* copepodid and adult biomass (r=-0.649, p<0.05, df=9, Fig. 3), and between female *S. tanganicae* GSI and *T. tenellus* (r=-0.723, p<0.05, df=9). In a similar vein, *S. tanganicae* GSI showed significant negative correlations with copepod biomass of the following month (male *S. tanganicae vs. T. simplex* copepodids and adults, and copepod nauplii: r=-0.687, p<0.05, Fig. 3; r=-0.696, p<0.05, Fig. 3, respectively, df=8; female *S. tanganicae vs. T. simplex* copepodids and adults, copepod nauplii and total copepods: r=-0.750, p~0.05, Fig. 3; r=-0.739, p<0.05, Fig. 3; r=-0.633, p<0.05; respectively, df=8).

GSI versus copepods of previous months - Reproductive investment in *S. tanganicae* was correlated with the food availability in the preceding months, as shown by significant correlations between average monthly GSI and copepod biomass three months earlier (male *S. tanganicae* vs. *M. aequatorialis* copepodids and adults: r=0.771, p<0.01 df=8; male *S. tanganicae* vs. total copepops: r=0.779, p<0.01 df=8, Fig. 6; female *S. tanganicae* vs. *M. aequatorialis* copepodids and adults: r=0.630, p~0.05, df=8). At this time lag, the GSI of female *L. miodon* also showed positive correlations with the biomass of *T. simplex* copepodids and adults (r= 0.800, p<0.01, df=8) as well as total copepods (r=0.702, p<0.05, df=8; Fig. 6). At other time lags and for the total copepod biomass integrated over preceding months, no significant correlations were observed (statistics not shown).

DISCUSSION

We investigated the timing of reproductive activities of two clupeid fishes (*S. tanganicae* and *L. miodon*) from the northern end of Lake Tanganyika (Bujumbura sub-basin) by comparing month-to-month fluctuations of the gonadosomatic index (GSI) with fluctuations in rainfall and the abundance of copepods. As copepods represent the main food source of larvae and juveniles of both species, but particularly *S. tanganicae* (Mannini et al., 1996; Lensu, 1998; Sarvala et al., 2002; Isumbisho et al., 2004), it was expected that the main period of reproductive activity (as indicated by a significant drop in GSI and in the percentage of ripe gonads) would precede or coincide with the rainy season, when the availability of copepods as a food source for larvae or juveniles is maximal (Chèné, 1975; De Iongh et al., 1983; Mgana et al., 2014). We could confirm this expectation for *S. tanganicae* in two different years (2004-2005 and 2007-2008), but only in one year for *L. miodon* (2004-2005). However, we found no indications that the fishes actively synchronize their reproductive activities with this optimal period (see below). Instead, correlations between GSI and copepod abundance suggested that reproductive activities are related to the availability of copepods in the preceding months.

Rainfall and copepods

Seasonal development of copepod biomass in 2007-2008 was closely associated with the rainfall pattern, at least partly because the rainfall brings new nutrients into the lake, facilitating phytoplankton production (see discussion in Mulimbwa et al., 2014). In particular, the increasing food supply in early wet season is likely to have a profound effect on copepod abundance (Narita et al., 1985; Mulimbwa, 1988, 1991; Kurki, et al., 1999). Overall, phytoplankton and zooplankton are assumed to be well correlated in Lake Tanganyika (Coulter, 1991). The realized zooplankton abundance, however, results from a balance between zooplankton production and its consumption by fish and invertebrate predators. For example, the low total copepod biomass noticed in dry seasons suggests a severe predation by *S. tanganicae* showing peak catches in July (Roest, 1992; Mulimbwa, 2006; Mulimbwa et al., 2014). The dominant factor causing the high total copepod biomasses in the rainy season was likely to be the availability of phytoplankton combined with a moderate predation pressure. Comparisons of the monthly changes in copepod abundance with historical studies are difficult due to different methodologies used (Rufli & Chapman 1976; Burgis, 1984), but the Lake Tanganyika Research (LTR) time series from the 1990s (Kurki, 1997) and the early 2000s (Mölsä et al., 2002) were based on compatible methods. In 1993-1996 and in 1999-2001, the seasonal changes in copepod biomass in the Bujumbura, Uvira and Kigoma sub-basins were highly variable with no consistent pattern relative to the dry and wet seasons (Kurki et al., 1999; Mölsä et al., 2002; Sarvala et al., unpublished). The seasonality of copepods as a food source for the pelagic planktivorous fish thus varies considerably from year to year and between areas.

Clupeid reproductive investment

One of the investigated clupeid species, *S. tanganicae*, showed the highest mean values of the gonadosomatic index (GSI) in the dry season. An abrupt decline indicated that the main spawning took place a few weeks before the rainy season. Although the onset of the rainy season is accompanied by a steep increase in total copepod biomass, we have no indications that *S. tanganicae* adjusts its spawning strategy to optimize the survival of its offspring. First, it is unclear what kind of trigger the fish would use to do so. Rainfall and associated increased turbidity would be one potential trigger, but the main spawning clearly occurred before the first rains. Second, at the time of spawning food availability was still low, and hence not beneficial to the larvae that would hatch in 2-3 days (Matthes, 1967) and likely start feeding within a week. Arguably, the timing of spawning could still be beneficial in a later developmental stage, but the benefit for the larvae as the most vulnerable stage would be larger. In the mid-1990s, Mannini et al. (1996) observed peak values for GSI at intervals of 3-4 months in female *S. tanganicae* in the north part of Lake Tanganyika. A similar periodicity in female *S. tanganicae* was observed in this study, although for both 2004-2005 and 2007-2008 the intervals seemed to approach five months. Length-frequency distributions for 2007-2008, however, clearly indicated four annual cohorts (Mulimbwa et al., 2014), but not all of those were equally strong, and it may be difficult to observe any significant changes in GSI related to the weaker cohorts.

In contrast to *S. tanganicae*, and especially in 2007-2008, the reproductive activity of *L. miodon* did not suggest a main spawning event, consistent with observations in the mid-1990s by Mannini et al. (1996). It hence seems that *L. miodon* is spreading its reproductive activities over longer periods of time. The reason for the variation between years remains unclear; the year 2007-2008 had lower rainfall than 2004-2005 (see Fig. 2A vs. 2D), but there were no significant differences in reproductive investment (results not shown). Repeated spawning of clupeids is an adaptation to an unstable aquatic ecosystem where considerable variation in fishing and natural mortalities causes some of the eggs to die (Coulter, 1961). The prolonged spawning provides stable replacement by successive cohorts. However, this is only possible when there is a long period of adequate food supply for the larvae. *L. miodon* might obtain such supply in the littoral zone, where food is abundant year-round (Coulter, 1991).

Reproductive investment vs rainfall and copepods

The year-to-year and spatial variation in the seasonality of copepods in Lake Tanganyika is expected to lead to corresponding variability in fish reproduction. In this study, the dependence of reproductive activities of both clupeids on the copepod food source was suggested by a positive correlation between GSI and the amount of copepods available three months before. Reproductive effort in both clupeids might therefore simply reflect the amount of available food in previous months. Conversely, but only for *S. tanganicae*, we observed a negative correlation between GSI and copepod abundance in the corresponding and following month. This suggests a predation effect by emerging *S. tanganicae* cohorts on the copepods. Although rainfall was also correlated with copepod abundance, this positive relationship was only observed in the concurrent month, consistent with the short generation times of planktonic copepods. In contrast, the negative effect of predation by *S. tanganicae* lasts longer as the fish are more long-lived and the total food consumption of a fish cohort likely peaks during the first few months of life (Mannini et al., 1996).

Changes in fishing pressure, climate change (including changes in rainfall, temperature and wind), or changes in environmental conditions in the drainage area (e.g. deforestation) might all lead to changes in the predator-prey dynamics between clupeids and copepods. Among these possible effects, changes in fishing pressure are relatively well documented (see Introduction). Overfishing in recent years might have decreased the effect of predation on copepods, such that correlations with rainfall and copepods should become predominant. This might explain why in the present situation of depleted fish stocks (e.g. Mulimbwa, 2006) we observed a clear correlation between rainfall and copepods, whereas this was not the case in the past (Kurki et al., 1999; Mölsä et al., 2002; Sarvala et al., unpublished) when the fish stocks were stronger and thus predation effects were much larger. Nevertheless, predation effects could still be observed even in our data.

CONCLUSIONS

Copepod zooplankton abundance was very high in the rainy season, providing an opportunity for larval clupeids to maximize survival rate. However, clupeid spawning activities did not seem to be adjusted to allow the larvae to optimally benefit from this food source. While *S. tanganicae* seemed to concentrate its reproductive activities at the end of the dry season, there was no well-defined spawning season in *L. miodon*. However, in both species the investment in reproduction seemed to depend on the availability of copepods three months earlier. We conclude that apart from some common elements, clupeid species may significantly differ in reproductive strategies. Further investigation should highlight the variation in clupeid reproductive activities across species and years, as well as the relationship with the abundance of zooplankton.

The observed differences between clupeid species are important from a management perspective. A long period of adequate food supply may be very important to support reproductive activities in species such as *L. miodon*, in order to maintain its intense exploitation at a sustainable level. This requires that the environmental conditions of the lake and its adjacent regions be preserved. As a littoral spawner (Matthes, 1967; Pearce, 1985; Coulter, 1970), *L. miodon* is particularly vulnerable to silting caused by soil erosion in the drainage area. Although the pelagically-spawning *S. tanganicae* is less susceptible to such changes, increased turbidity during its peak reproductive activities in the rainy season might be harmful. Thus even this species may benefit from reforestation that would help to preserve and restore the natural equilibrium of the lake, promoting the survival and growth of its copepod prey.

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Axanthism in amphibians: A review and the first record in the widespread toad of the *Bufotes viridis* complex (Anura: Bufonidae)

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ABSTRACT. Axanthism in amphibians is a relatively rare color aberration reported less often than leucism or albinism. It is caused by lack of specific types of pigment cells in the skin, namely xanthophores, erythrophores, and iridophores. Here, we present the first case of occurrence of this aberration in a widely distributed toad of the green toad (*Bufotes viridis*) complex and provide an extensive review of axanthism occurrence in amphibians. So far it has been reported in more than 20 species from nine families of amphibians with the highest occurrence in the family Ranidae.

KEY WORDS: amphibia, *Bufo viridis*, color aberration, Slovakia.

INTRODUCTION

Coloration plays an important role in various aspects of animal life history. In many species of amphibians and reptiles cryptic or aposematic coloration enhances protection against visual predators, while at the same time it can provide valuable information to cognates about sex, fitness, maturity or availability for reproduction (VITT & CALDWELL, 2009). Therefore natural selection usually eliminates any aberrations that occur (ANDRÉN & NILSON, 1981). However, there are several examples when specimens or even populations that are characterized by aberrant coloration, can survive and reproduce, e.g. melanic specimens of the common lizard *Zootoca vivipara* or an albinotic population of the snake *Elaphe climacophora* (SCHULZ, 1996; GVOŽDÍK, 1999; JAMBRICH & JANDZIK, 2012). Changes in coloration are also often related to life in the dark, e.g. in caves, as is known in the Mexican tetra *Astyanax mexicanus* (MITCHELL et al., 1977; WILKENS, 1988), whose surface populations are normally pigmented, while the cave populations lack pigmentation. Another such example is the olm *Proteus anguinus*, a troglobiont urodele known in two forms: either completely unpigmented or with black pigmentation (SKET & ARNTZEN, 1994).

Color aberrations that have been described in vertebrates are albinism, amelanism, axanthism, erythrism, hypomelanism, leucism, melanism and piebaldism, though this list is not exhaustive and nomenclature is not consensual (BECHTEL, 1995). In wild amphibians the literature reports albinism (white or yellowish body color and red eyes) and leucism (white or pinkish body color, dark eyes) as the most common forms of aberrant coloration (e.g. WERNER, 1893; SMALLCOMBE, 1949; CAMPANNA, 1973; DYRKACZ, 1981; BECHTEL, 1995; MIKULÍČEK et al., 2001; THOMAS ET AL., 2002; PABIJAN et al., 2004; LÓPEZ & GHIRARDI, 2011; ESCORIZA, 2012). They usually result from gene mutations affecting development and distribution of chromatophores and/or skin pigment production (Duellman & Trueb, 1994; Bechtel, 1995).

Axanthism characterized by blue, bluish or generally grey, dark body color, very dark patterns, and dark eyes, is one of the least known aberrations, though it is presumably as widespread as albinism (Bechtel, 1995). Skin of axanthic animals lacks xanthophores (Browder, 1968), erythrophores, and iridophores, which normally produce yellow and orange, red, and light-reflecting and scattering pigments, respectively. In some cases, the xanthophores might be unable to produce the pigment, despite the fact they are present (Berns & Narayan, 1970). The lack of iridophores typically results in duller and darker overall coloration. In contrast to the skin, the eyes of axanthic specimens may contain iridophores and melanophores (rarely also xanthophores; Bechtel, 1995; Frost-Mason & Mason, 1996). So far, axanthism has been reported either in a partial or a complete form in several species of amphibians (e.g. Liu, 1931; Dubois & Vachard, 1971; Dubois, 1979; Juszczyk, 1987; Bechtel, 1995; Vlček, 2003, 2008; Niccoli, 2013; for other references and detailed information see Table 1).

AXANTHISM IN THE GREEN TOAD *BUFOTES VIRIDIS*

The taxonomically complicated species complex of green toads [*Bufotes viridis* (Laurenti, 1768), complex; also known as *Bufo viridis* in traditional taxonomy] includes at least 14 morphologically similar species distributed across Europe, Asia and Northern Africa (Stöck et al., 2006, 2008; Frost, 2014). Coloration and pattern of these toads varies across their distribution range, but generally the background coloration is light to dark brown, covered with darker green patches creating a camouflage pattern. Tiny red spots might be present on various parts of the body. The belly is usually pale without a pattern and the iris is yellow or yellowish (Arnold & Ovenden, 2002). Interestingly, despite the variation in tint or intensity of both background color and pattern, color aberrations are relatively rare within this species complex. To the best of our knowledge, only albinism (Flindt, 1985; Andrä, 2011), erythrism (Lanza & Canestrelli, 2002) and retinal depigmentation (Engelmann & Obst, 1976) have been reported so far.

Here, we report an axanthic juvenile specimen (approx. SVL 30 mm) of *B. viridis*, encountered in Pezinok, SW Slovakia (48.31993° N 17.24085° E; June 28, 2009). The specimen was overall very dark with some bluish tint on the belly. Brighter parts were confined to the area around mouth, throat and eyes and some brighter spots were also observed on parotid glands, dorsum, and hind limbs. Skin patches that normally form a camouflage pattern were also darker, though the contrast between them and the dark background was hardly detectable. Some poison gland openings had white or yellow tips, and tiny yellowish spots were also present on flanks anterior to hind limbs (Fig. 1). Between 35 and 50 normally pigmented specimens of *B. viridis* were observed in the area at the same time. From the total number of about 150 observed specimens in the season of 2009, only the one reported here was axanthic (0.7 %).

AXANTHISM IN AMPHIBIANS

Axanthism remains a rarely observed and reported color aberration in amphibians when compared to other color aberrations (see Dubois, 1979; Bechtel, 1995). In partially axanthic specimens it is easy to overlook this aberration, and completely axanthic specimens can easily be confused with melanic specimens. These, similarly to the axanthic ones, are also dark and have a somewhat disrupted pattern. Distinction between melanism and axanthism is possible based on the slightly lighter coloration of axanthic specimens, in which also the pattern is typically discernible. This is also the case of the green toad specimen reported herein. The anatomical explanation has been offered from studies on axolotls, in which melanic specimens lack both xanthophores and iridophores in their skin, but unlike axanthic specimens, they also over-proliferate melanophores resulting in much darker coloration (FROST-MASON & MASON, 1996). According to our summarized literature data (see Table 1), three basic types of axanthism can be recognized in amphibians: (i) complete or partial blue body coloration (mainly in Ranidae, see Table 1 and references therein), (ii) complete or partial grayish or dark body coloration, (iii) normal body coloration with black eyes. These three types are not mutually exclusive and combinations can occur (e.g. blue coloration with black eyes).

CAUSES OF AXANTHISM

Although the genetic background of pigment cell formation in amphibians is relatively well known (NEVO, 1973; FROST-MASON & MASON, 1996; HOFFMAN & BLOUIN, 2000), proximate causes of axanthism remain unknown. Besides genetic mechanisms, environmental causes cannot be excluded, such as temperature fluctuations, food quality, parasitism or environmental pollution (cf. DUBOIS, 1979; VERSHININ, 2004; CABALLERO et al., 2012). Interestingly, many observations of axanthic specimens (mainly in the family Ranidae; see Table 1) were reported from 1960-1990s from urban areas of industrial countries with potentially high levels of environmental pollution (cf. DANDOVÁ et al., 1995). However, in some cases the darker coloration can offer an adaptive advantage, e.g. in thermoregulation, which is particularly important in ectothermic vertebrates (e.g. melanic *Vipera berus*; ANDRÉN & NILSON, 1981). Conversely, loss of cryptic or aposematic pattern and coloration might lead to an increased conspicuousness and decreased intensity of warning signals, respectively, and thus a higher predation risk (CHILDS, 1953; ANDRÉN & NILSON, 1981). It is possible that in axanthic specimens a genetic relationship exists between

Fig. 1 – Axanthic specimen of the green toad *Bufotes viridis* from Slovakia. A: dorsal view; B: lateral view; C–D: comparison with normally colored specimen of the same size and from the same locality.

TABLE 1

A summary of the axanthism occurrence in amphibians.

Species	**N**	**Locality**	**Dorsal pattern**	**Background color**	**Eye color**	**Stage**	**Sex**	**Habitat**	**References**
Ambystomatidae									
Ambystoma mexicanum	-	laboratory	dark	grey	black	larvae, adult	-	-	Frost et al., 1984
Salamandridae									
Lissotriton helveticus	1	Fontainebleau, France	visible	normal	black	adult	M	forest pond	Dubois et al., 1973
Taricha granulosa	1	Corvallis, Oregon, USA	any indication of the yellow-orange pigment	dorsal deep blackish-brown, ventral slightly lighter blackish-brown	black	adult	M	artificial pond	Livezey, 1960
Alytidae									
Alytes obstetricans	?	Arteixo, Spain	-	dark, light ventrally	black	juvenile	-	-	Galan et al., 1990
Bufonidae									
Anaxyrus woodhousii	1	North Carolina, USA	unspotted chest, warts on each of the largest dark spots	blue	-	-	-	backyard, urban area	Bechtel, 1995
Bufo bufo	3	Paris region, France	-	brown-grayish-pink abnormal pigmentation	black-brown	2 tadpoles, 1 adult	M (adult)	-	Dubois, 1969
Bufotes viridis	1	Pezinok, Slovakia	dark, partly visible	dark	black	juvenile	-	urban area	this study
Craugastoridae									
Craugastor phasma	1	Las Tablas, Costa Rica	not visible, fifteen to twenty black spots	gray white	black	adult	F	rocky stream bank	Lips & Savage, 1996
Dicroglossidae									
Euphlyctis cyanophlyctis	2	Yangdi Khola, Suikhet, Nepal	partly visible	grayish	black	adult	M	urban area	Dubois, 1976
Hylidae									
Acris crepitans	1	Harrison Lake, Charles City, USA	-	mottled pattern of tan/grey, green, and blue	-	juvenile	-	lake	Niccoli, 2013
Hyla arborea	1	Apetlon, Austria	-	dark	black	adult	-	-	Hinz, 1976
Hyla cinerea	1	Brazos County, Texas, USA	-	dark gray except green head and shoulder	black	adult	F	farm pond	Cain & Utesch, 1976
Hyla japonica	1	Konosu, Japan	-	blue	-	juvenile	F	-	Nishioka & Ueda, 1985a
Hyla japonica	1	Tottori, Japan	-	blue	-	juvenile	F	-	Nishioka & Ueda, 1985a
Hyla japonica	1	Hesaka, Japan	-	dark grayish-brown; dark grayish-olive	black	-	F	-	Nishioka & Ueda, 1985d
Hyla japonica	3	Yachiyo-cho, Japan	-	dark grayish-brown; dark grayish-olive	black	1 juvenile, 2 adult	2F, 1M	greenhouse	Nishioka & Ueda, 1985d

Ranidae									
Pelophylax esculentus	1	Poland	dark, partly visible	grayish	black	adult	-	-	Juszczyk, 1987
Pelophylax esculentus	1	Havířov, Czech Republic	normal, visible	normal	black	adult	M	natural wetlands	Vlček, 2003
Pelophylax esculentus	1	Oldenburg, Germany	dark, visible	normal	black	adult	F	fishpond	Fischer, 1999
Pelophylax lessonae	1	Žermanice, Czech Republic	partly visible	grayish	black	adult	M	flooded mine	Vlček, 2008
Pelophylax lessonae	1	Svatý Kříž, W Bohemia, Czech Republic	dark, partly visible	dark	black	adult	M	fishpond, urban area	Dandová et al., 1995
Pelophylax nigromaculatus	1	Wei-hsiu Yuan, China	-	blue	-	-	-	lotus pond	Liu, 1931
Pelophylax porosus	1	Maki-cho, Nishikambara-gun, Japan	yellowish dorso-lateral and dorso-median stripes	semitransparent, blackish	light yellowish-brown	adult	F	-	Nishioka & Ueda, 1985c
Pelophylax plancyi	7	Wei-hsiu Yuan, China	-	blue	-	subadults	-	lotus pond	Liu, 1931
Lithobates catesbeianus	1	Kentucky, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	69	MW Wisconsin, SE Minnesota, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	31	scattered throughout Maine, Vermont, Massachusetts, New York, New Jersey (USA), SE Canada	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	1	Virginia, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	2	USA	-	blue and green	-	-	-	-	Berns & Narayan, 1970
Lithobates pipiens	1	Sapelo Island, Georgia, USA	partly visible	light sky-blue, green, dark brown	-	adult	F	-	Martof, 1964
Lithobates pipiens	3	MW Wisconsin, SE Minnesota, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	6	scattered throughout Maine, Vermont, Massachusetts, New York, New Jersey (USA), SE Canada	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	1	Georgia, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	1	Broadus, Montana, USA	visible, normal	Blue coloration over the back from the tips of the external nares to the anus	-	-	-	-	Black, 1967
Lithobates pipiens	-	laboratory	dark, visible	dark	black	tadpoles, adult	-	-	Richards et al., 1969
Lithobates sylvaticus	15	Innoko, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	118	Kenai, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	20	Tetlin, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	1	Yukon Delta, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Rhacophoridae									
Rhacophorus schlegelii	8	Okuyama, Ashiya, Hyogo, Japan	-	dark brown	black	adult	7F, 1M	rice field	Nishioka & Ueda, 1985b

the lack of certain types of chromatophores and harmful mutations, decreasing the chance of individual survival and/or genetic fixation of this aberration at the population level. All of this can underlie the relative rarity of this aberration in general and in adult specimens in particular (DUBOIS, 1979).

TAXONOMIC DISTRIBUTION OF AXANTHISM IN AMPHIBIANS AND FREQUENCY OF OCCURRENCE

Despite relatively broad taxonomic distribution of axanthism in amphibians (9 families, 23 species of the Americas, Asia and Europe, see Table 1), we can consider it a rare aberration. The largest number of examples was reported from the family Ranidae (23 cases from 9 species; cf. BERNS & UHLER, 1966; DUBOIS & VACHARD, 1971; DUBOIS, 1979) and it seems to be far more common in frogs than in any other group of amphibians (see Table 1). The data on intraspecific or intrapopulation frequencies are scarce and difficult to obtain. Berns and Uhler (1966) reported frequencies within different localities, finding two blue specimens out of 1000 normal frogs (0.2%) and 22 out of 7000 (0.3%) frogs for *Lithobates clamitans*. However, Dubois (1979) reported significantly higher frequencies – 101 (8.5%) of blue post-metamorphic specimens in 1186 *Pelophylax* sp. from France and four (3.2%) among 126 specimens from Iran, while the same author found only eight (0.2%) black-eyed frogs among 4651 *Pelophylax* sp. and one (0.1%) in 777 *Bufo bufo* (DUBOIS, 1969; DUBOIS, 1979).

Data on frequencies of other color aberrations occurring in amphibians (albinism, leucism, melanism etc.) are scattered or completely lacking. The literature record of albinism and leucism is extensive (see e.g. WERNER, 1893; SMALLCOMBE, 1949; BRAME, 1962; CAMPANNA, 1973; DUBOIS, 1979; DYRKACZ, 1981; BECHTEL, 1995; MIKULÍČEK et al., 2001; MITCHELL, 2002; PABIJAN ET AL., 2004; SPADOLA & INSACCO, 2010; LÓPEZ & GHIRARDI, 2011; MODESTI et al., 2011; TOLEDO et al., 2011; ESCORIZA, 2012; KEELY & MALDONADO, 2013 and references therein), so it can be easily assumed that these two aberrations are more frequent than axanthism in natural populations. On the other hand, albinotic or leucistic specimens are more conspicuous and more interesting to record, while axanthic specimens could be misidentified as melanic or entirely overlooked, so this comparison should be treated with care. Contrary to albinism and leucism, the frequency of melanism and piebaldism seems to be lower than that of axanthism in amphibians (cf. BECHTEL, 1995).

Axanthism represents an interesting color aberration of amphibians that deserves more attention. So far, it is only logical that most cases of its occurrence are known from countries where most of the herpetological research has been carried out, i.e. North America and Europe. With more studies appearing from the other parts of the world, we can expect that both taxonomic and geographical distribution of axanthism will expand.

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Inter-individual variability in reproductive success and somatic growth in *Cichlasoma dimerus* (Heckel, 1840)

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ABSTRACT. Environmental factors and social interactions are known to affect somatic growth and reproduction in teleost fish. It has been described for *Cichlasoma dimerus* that only one pair is formed under a wide range of laboratory conditions. However, this was not observed in tanks composed of three males and three females, where multiple pair formation occurred. Thus, our objective was to evaluate somatic growth and reproductive performance in *C. dimerus* under this particular condition, in which more than one pair is expected to be formed. A clear sexual growth dimorphism, with males growing faster than females, and multiple pair formation, sometimes simultaneously, were observed. Both features were absent in previous studies with other aquaria structures. Additionally, there was a significant association between reproductive events and body size, where the bigger the fish, both male and female, the higher the number of reproductive events. Despite the sexual growth dimorphism, no differences were observed between males and females in IGF-I and GHR2 mRNA levels. The results obtained for this social species show a high inter-individual variability in the aquaria in regard to reproductive success and growth. This may have implications on experimental design, where a low level of heterogeneity between fish is desirable. If this variability is not taken into account, possible treatment effects may not be detected.

KEY WORDS: Body size, cichlids, growth dimorphism, reproductive events.

INTRODUCTION

In teleost fish somatic growth is affected by environmental factors such as variations in the photoperiod (VERA CRUZ & BROWN, 2009), temperature (GABILLARD et al., 2005), social interactions and food availability (VERA CRUZ & BROWN, 2007, 2009). Somatic growth is defined as a change in size over time. This can be assessed by measuring differences in length and/or weight, implying different physiological processes (BECKMAN, 2011). Therefore, somatic growth is presented in literature by diverse parameters such as difference between final and initial length or body weight, somatic growth rate and condition factor among others. Growth is regulated by the growth hormone (GH)/ insulin-like growth factor-I (IGF-I) axis (DUAN, 1998; BJÖRNSSON et al., 2002; WOOD et al., 2005; REINECKE, 2010). GH acts directly on certain tissues and indirectly via endocrine or local IGF-I production (ROUSSEAU & DUFOUR, 2007). GH exerts its actions by binding to specific receptors (GHRs). Two GHR types have been described: GHR1, which has been proposed to be the somatolactin receptor, and GHR2, the GH receptor (FUKADA et al., 2004, 2005). The liver is the primary source of circulating IGF-I, but its expression is well documented in other extra-hepatic tissues with a possible local function (REINDL & SHERIDAN, 2012). BECKMAN (2011) discusses the use of IGF-I as a possible indicator of somatic growth in fish and concludes that before using IGF-I as a growth index in a given situation, the concordance between IGF-I and growth should be tested in that particular situation.

Somatic growth heterogeneity is a characteristic present in many teleost fish (FERNANDES & VOLPATO, 1993; STEFÁNSSON et al., 2000; VERA CRUZ & BROWN, 2007). Social hierarchies, differences in feeding, and gender are factors that could affect somatic growth rates (JOBLING & REINSNES, 1986; RILEY et al., 2002a; MANDIKI et al., 2004; VERA CRUZ & BROWN, 2007; JI et al., 2011). Many vertebrate species establish social hierarchies with dominant and subordinate individuals. Several studies carried out in fishes show that dominant individuals aggressively defend their territory and are reproductively active (ELLIS, 1995; FERNALD & HIRATA, 1977; SAPOLSKY, 2005; FERNALD, 2009; RYDER et al., 2009), with some social species showing a relationship between agonistic interactions and size (ABBOT et al., 1985; BEAUGRAND et al., 1996). Besides, it has been observed that social interactions and feeding hierarchies result in individual growth rate variations (JOBLING & REINSNES, 1986; RILEY et al., 2002a; MANDIKI et al., 2004; VERA CRUZ & BROWN, 2007; JI et al., 2011). In addition, it is well-known that certain fish species exhibit sexual dimorphism in somatic growth. For instance, in some species males grow faster than females, such as in the tilapia *Oreochromis mossambicus* (PETERS, 1852) (RILEY et al*.*, 2002a), and in others females grow faster than males, such as half-smooth tongue sole *Cynoglossus semilaevis* (GÜNTHER, 1873) (JI et al., 2011) or Eurasian perch *Perca fluviatilis* (LINNAEUS, 1758) (MANDIKI et al., 2004). Several studies suggest that gonadal steroid hormones modulate the GH-IGF-I axis in fishes (RILEY et al., 2002b, ARSENAULT et al., 2004; LARSEN et al., 2004; MANDIKI et al., 2004; DAVIS et al., 2007, 2008).

Cichlasoma dimerus (HECKEL, 1840) is a South American cichlid fish that exhibits high growth rates under laboratory conditions. As many cichlids do, *C. dimerus* displays highly organized breeding activities, as can be observed in the laboratory. The dominant pair will aggressively defend the prospective spawning site (usually a flat stone) and will start to display stereotyped pre-spawning activities (PANDOLFI et al., 2009). In a pair, the male is bigger than the female. The somatic growth rate and the reproductive performance in a time period depend on the condition in which the fish are maintained. For example, in tanks composed of four males and four females only one pair is formed between a female and the biggest male in the aquaria (preliminary results of our laboratory and ALONSO et al., 2011). In these conditions we did not observe differences in somatic growth rate between genders. On the other hand, in tanks composed of three males and three females, multiple pairs are established (previous observation). Thereby, the aim of the present study was to evaluate somatic growth and sexual performance in fish maintained in tanks where more than a pair is expected to be formed.

MATERIALS AND METHODS

Animals

Adult *C. dimerus* were collected from "Esteros del Riachuelo", Corrientes, Argentina (27°12'50"S, 58°11'50"W), transferred to the laboratory and acclimated in fresh water tanks (400l) under stable conditions of temperature (25 ± 3)°C and photoperiod (14 hours light:10 hours dark). Pairs of *C. dimerus* established in these tanks were withdrawn and maintained together in individual aquaria until their use in the experiment. During this time, fish were fed daily with commercial pellets (Tetra Pond Variety Blend, Tetra). A pair was considered formed after a reproductive event occurred. Suitable actions were followed to minimize pain or discomfort to fish. Principles of laboratory animal care (Guidelines on the care and the use of fishes in research, teaching and testing, Canadian Council on Animal Care, 2005) were adopted. Experiments comply with the approval of Comisión Institucional para el Cuidado y Uso de Animales de Laboratorio, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina (protocol number 26).

Experimental protocol

The experiment was carried out from November 2010 to May 2014. Each replication (n = 5) consisted of three pairs (the ones previously obtained) placed in a 400L aquarium (density = 15 fish/m^3) containing gravel, artificial plants and some slabs, under the same temperature and photoperiod conditions as described above. Initial body mass (BM, males: 18.57 ± 1.1 g; females: 14.77 ± 1.08 g) and total length (L_T, males: 9.87 ± 0.19 cm; females: 9.20 ± 0.24 cm) were measured (P = 0.07 after MANOVA for differences in initial L_T and BM) immediately preceding the experiment. Animals were kept for two months under these experimental conditions and daily fed with the same commercial pellets. During this period, they were individually identified by their sizes and the particular spot pattern in their fins. Every day during 30 minutes, between 11-12 AM, feeding behaviour, reproductive events and hierarchy status were visually registered. In addition, several times during the course of each experiment, a video recording was performed. Feeding behaviour, rather than food intake, was evaluated as the intention of the fish to catch a pellet. When these fish take a pellet into their mouths, they spit it out many times. Therefore it is not possible to directly quantify the amount of food actually ingested.

In order to evaluate a possible relationship between the number of reproductive events (defined as the egg spawning and the posterior fertilization by the males' sperm release) and initial body size, each fish per tank was classified according to its sex and initial BM or L_T. The smallest fish in a tank was ranked as number 1, the next one as number 2 and the biggest one as number 3. This classification was carried out for males and females separately.

Tissue sample and transcripts quantification

After a two months span, BM and L_T were measured. Fish were anesthetized with benzocaine 0.1%, euthanized by decapitation, and livers collected. As indicators of the nutritional status, hepatosomatic index (I_H = organ mass/body mass*100) and condition factor (CF = BM*$(L_T)^{-3}$*100) were calculated. For evaluating growth in each fish during the two months experiment ΔL_T (final L_T - initial L_T), ΔBM (final BM - initial BM) and specific growth rate (SGR) were calculated. SGR is defined as Ln(Xf/Xi)* t^{-1}*1000, where t is time measured in days and Xf and Xi denote BM or L_T at final and initial moment of the experiment, respectively.

Total liver RNA of 16 fish from three tanks was obtained by TRIzol® (Invitrogen) following manufacturer's instructions, and the corresponding cDNA was synthesized in order to quantify the different transcripts by quantitative real-time polymerase chain reaction (RT-qPCR). RT-qPCRs for IGF-I, GHR2 and acidic ribosomal phosphoprotein P0 (ARP) (reference gene) were performed by using FastStart Universal SyBR green Master (ROCHE) with a mixture of forward and reverse specific primers (Table 1) and 2.5μl of cDNA template per tube. The RT-qPCR protocol was as follows: 10min of denaturation at 95°C and 40 cycles of 95°C for 15 sec, 58°C for 30 sec and 72°C for 20 sec.

TABLE 1

Primers used for Real Time qPCR.

Name	Sequence (5' to 3')
GHR2 forward	ACTGCTCTCCACTCTCAATTG
GHR2 reverse	AAAGGTGATGGTTCTGGGTC
IGF-I forward	CTCCCAAGATTTCTCGCTCTG
IGF-I reverse	CCCTTCTCCGCTTTACTAACC
ARP forward	ACTGTGGGAGCAGACAATG
ARP reverse	TCCAGTGCAGGATTGTTCTC

Statistical analysis

Differences in mean total length (ΔL_T) and body mass (ΔBM) between genders were evaluated by using a Randomized Block Design (RBD) ANOVA considering the fixed factor gender and the random factor experimental replication (*i.e*, each tank). In order to discard any influence of the initial body size on growth performance between genders, Pearson's correlations between initial L_T against ΔL_T and BM against ΔBM for each sex were performed. For statistical analysis a single value per sex was used by averaging three fish per tank to avoid pseudo-replication. A Spearman's correlation was run to determine the relationship between reproductive events and body size ranks.

Raw qPCR data were submitted to LinRegPCR software processing in order to obtain initial fluorescence values per sample for subsequent analysis (RAMAKERS et al., 2003; RUIJTER et al., 2009). Pearson's correlations were used to evaluate possible associations between IGF-I mRNA levels and ΔBM, ΔL_T and SGRs. IGF-I and GHR2 mRNA levels between genders were analyzed by using a RBD ANOVA as it was done for morphometric parameters.

Values are expressed as means ± S.E.M. Statistical tests were considered significant when p-values were less than 0.05 by using InfoStat/L software. Sequential Bonferroni's correction was applied to keep the type I family-wise error rate at 0.05 level.

RESULTS

Morphometric parameters

ΔL_T and ΔBM were significantly higher in males than in females (0.60 ± 0.09 cm *vs.* 0.16 ± 0.07 cm, P = 0.0034) and (4.32 ± 0.95g *vs.* 0.90 ± 0.26g, P = 0.0285), respectively (Fig. 1a,b). For the sizes assessed in this experiment, we did not find any significant correlations between initial body size and ΔL_T or ΔBM (Pearson's correlation, P>0.05). In addition, SGR(L_T) was significantly higher in males than in females (1.36 ± 0.32 *vs.* 0.32 ± 0.15 respectively, P = 0.0194). Although ΔBM was higher in males than in females, the differences for the SGR(BM) were not statistically significant (3.49 ± 1.46 *vs.* 0.96 ± 0.31 respectively, P = 0.1298). Even though females had a higher I_H, the difference between genders was not significant (males: 1.37 ± 0.11%

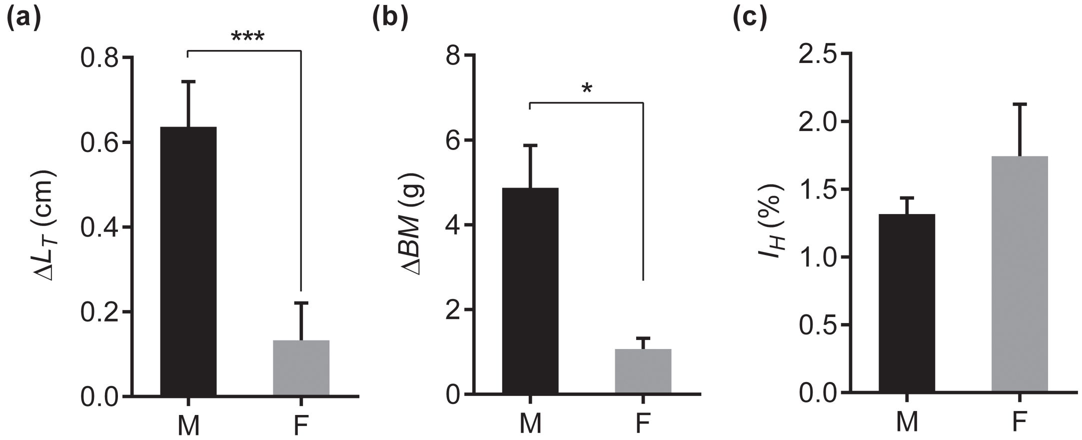

Fig. 1. – Morphometric parameters in three-pair tanks. Vertical bars represent the mean gain in (a) total length (ΔL_T), (b), body mass (ΔBM) and (c) hepatosomatic index (I_H). M: males; F: females. Results are presented as mean ± SEM. ***P<0.001.

vs females: 1.78 ± 0.30%, P = 0.3146) (Fig. 1c) nor was it for CF (males: 1.81 ± 0.08 *vs* females: 1.87 ± 0.11, P = 0.7579).

Reproductive performance

As expected for this species, we observed aggressive behaviour of the reproductive pair defending the spawning site. This behaviour continued during the reproductive event and the period of parental care.

In all the replications, as was expected from previous observations, more than one pair was observed during the experimental period. Those pairs were not stable, which means that after the reproductive event a new pair was formed, including one or both of these individuals. On some occasions, two pairs were present simultaneously. There was a strong positive monotonic correlation between reproductive events and the initial body size ranking for males (Spearman's correlation, L_T ranking: r_s = 0.78, P = 0.0011; BM ranking: r_s = 0.85, P = 0.0001) and females (Spearman's correlation, L_T ranking: r_s = 0.69, P = 0.0048; BM ranking: r_s = 0.69, P = 0.0048). The greater number of reproductive events was associated with the larger fish present in every tank for both genders (Fig. 2). In all the replications, the first pair involved the biggest male. Initial pairs, established before the beginning of the experiment, were not necessarily maintained.

Additionally, video recordings suggested no difference in feeding behaviour among fish of the same tank. Even during the reproductive event and parental care, the pair showed similar feeding behaviour to the other fish in the tank.

Hepatic IGF-I and GHR2 mRNA expression

There was no difference in hepatic IGF-I mRNA levels (P>0.05, Fig. 3a) nor in GHR2 mRNA levels (P>0.05, Fig. 3b) between genders. No correlations between hepatic IGF-I mRNA levels and ΔBM, ΔL_T and SGR were observed (Pearson's correlation, P>0.05).

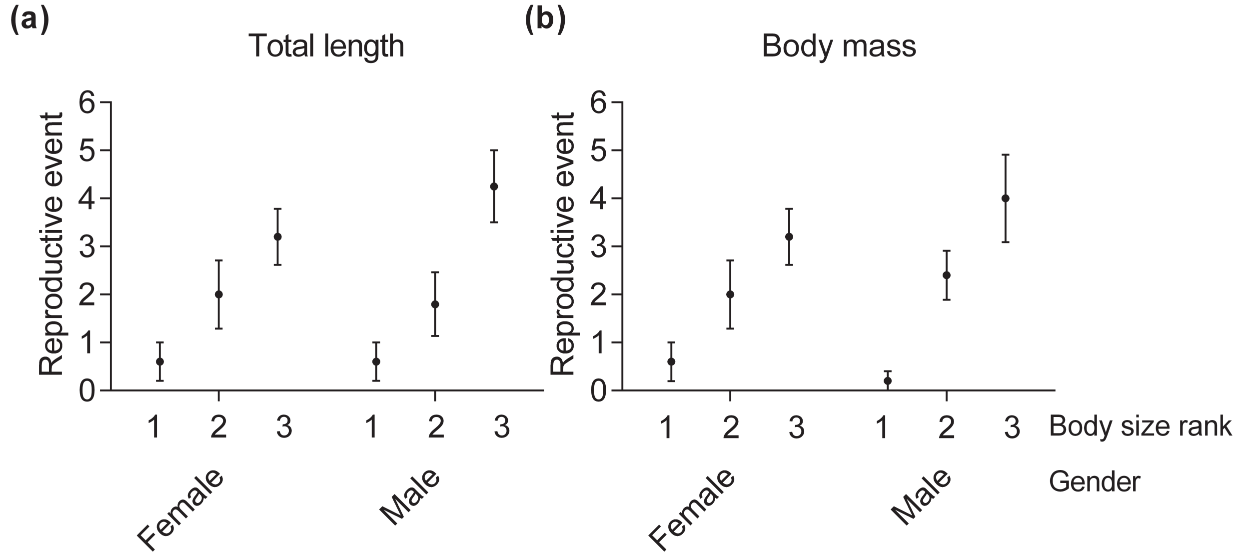

Fig. 2. – Relationship between reproductive events and the body size ranks. For both genders a greater number of reproductive events was associated with the larger fish present in every tank. Spearman's correlation was significant for total length (a) and body mass (b) both in males and females. Fish were ordered according to body size: 1: small; 2: intermediate; 3: big. Results are presented as mean ± SEM

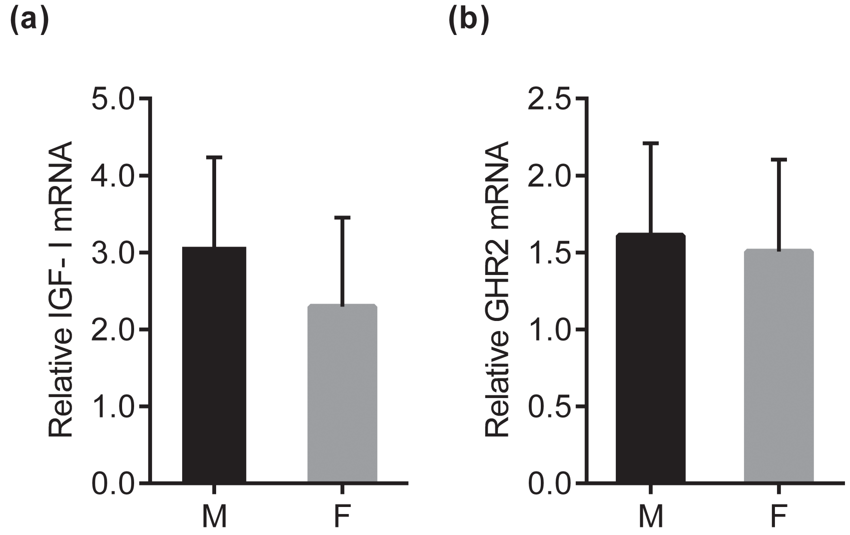

Fig. 3. – Hepatic IGF-I (a) and GHR2 (b) mRNA levels in males and females from three-pair tanks. Data were normalized using ARP as a reference gene and presented as a mean ± SEM. No differences were observed in IGF-I and GHR2 mRNA levels between genders after an RBD ANOVA test. M: males; F: females.

DISCUSSION

Heterogeneous somatic growth is a common characteristic in fish populations (Fernandes & Volpato, 1993; Stefánsson et al., 2000; Vera Cruz et al., 2007). For instance, differences in somatic growth between dominants and subordinates (Koebele, 1985; Vera Cruz & Brown, 2007) or between genders (Riley et al., 2002a; Davis et al., 2008) have been described for many cichlid fish species. In this study, sexual growth dimorphism was clearly demonstrated by evaluating the difference between sexes in body mass and total length gain after two months of experiment. This comparison showed that males grew more than females. However, as ΔBM and ΔL_T can be influenced by their values at the beginning of the experiment, we also compared their SGR values. These results are in line with those obtained with ΔBM and ΔL_T, showing that males display higher specific growth rates, statistically significant at least regarding length, compared to females. Additionally, we demonstrated that for the fish sizes used in this study, there was no relationship between the initial body size and ΔBM or ΔL_T for each sex, which therefore excludes the possibility of any influence of the initial body size on growth performance. Taken together, these results give strong evidence for sexual growth dimorphism in *C. dimerus.*

A role of steroids in the promotion of somatic growth has been studied in several species. In fishes in which males grow more than females, administration of androgens promotes growth and an increment in IGF-I (Riley et al., 2002a, b) whereas estradiol (E2) decreases it. In contrast, in those fishes in which females grow more than males, E2 would promote growth (Malison et al., 1985; Malison et al., 1988; Hayward et al., 2001) and also an increase in IGF-I (Goetz et al., 2009). Additionally, in Eurasian perch *P. fluviatilis*, androgens produced a decrease in growth (Mandiki et al., 2004). In the present study fish were in different reproductive states at the end of the experiment; gonodal steroids were therefore not measured due to the high variability expected. The question is, why was a clear sexual growth dimorphism observed in our experimental design? One possibility is that the sexual growth dimorphism was due to differences in food intake between males and females. However, this scenario seems to be unlikely as no apparent differences in feeding behaviour were observed. Moreover, the nutritional status parameters, hepatosomatic index and condition factor, were not different between genders. In our experiments fish were fed daily and food availability was never restricted, which should exclude the possibility of inequality in food availability. Another possibility could be a differential feed efficiency conversion between males and females and/or a bigger requirement of metabolic energy for reproduction in females, which would impact negatively on growth rate (MANDIKI et al., 2004). Specific experiments would be necessary to elucidate if the sexual growth dimorphism is due to differences in food intake and/or feed efficiency conversion.

Additionally, reproductive performance also depends on the tank conditions. Multiple pair formation was observed when three pairs were placed in the aquaria, a feature absent in four-pair tanks (preliminary results of our laboratory and ALONSO et al., 2011). The first pair formed always included the biggest male. Interestingly, in all tanks a strong correlation between reproductive events and body size was observed, where the bigger the fish, both male and female, the higher the number of reproductive events. To our knowledge this is the first report describing this association. In other reports, a positive correlation was observed between body size and fecundity success, but in those cases fecundity success was inferred as testis mass (PUJORAL et al., 2012), number of eggs per ovary (HOSSAIN et al., 2012) and sperm content (WATANABE et al., 2008). While gonadal parameters are widely used as indicators for fecundity, this does not necessarily imply new offspring because it depends on other events such as pair establishment and quality of the gametes among others. Unlike those approaches, our results show strong evidence on this issue.

In view of the somatic growth dimorphism observed, we investigated if there were changes in somatic growth axis that could explain this morphometric result. Unfortunately we could not measure GH expression in all the pituitaries due to technical issues during RNA extraction. In order to test if in *C. dimerus* a positive relationship exists between hepatic IGF-I mRNA and somatic growth, these parameters were compared in females and males. No correlation was observed between growth and IGF-I levels. Our results are in accordance with those obtained in chinook salmon *Oncorhynchus tshawytscha* (WALBAUM, 1792) and barramundi *Lates calcarifer* (BLOCH, 1790) (SILVERSTEIN et al., 1998; NANKERVIS et al., 2000). As summarized by Beckman (2011, p. 236-237), there are different relationships between IGF-I levels and somatic growth. In the present work hepatic IGF-I mRNA was measured at the end of the experiment *i.e.* at a specific time point. Unfortunately, it was impossible to measure IGF-I plasma levels due to the small plasma sample obtained. A possible scenario would have been to measure plasma IGF-I during the experiment (longitudinal design) but this kind of experimental design would have impacted negatively on the reproductive behaviour. Also, another component of the somatic growth axis, GHR2 was measured. No differences were observed in hepatic GHR2 mRNA levels, in agreement with no variations in IGF-I between genders. This result differs from that obtained in the Mozambique tilapia *O. mossambicus* (DAVIS et al., 2008) where males, which present greater somatic growth rates than females, showed higher level expression for both GHR1 and GHR2 mRNA receptors in the liver. Although in *C. dimerus* there were no differences at the expression level, possible differences at the protein level cannot be ruled out. More studies including measurements of plasma IGF-I and analysis of hepatic GHR2 content are necessary to check this out.

CONCLUSIONS

In summary, this work shows clear sexual growth dimorphism in fish held in tanks containing three males and three females, and a significant association between reproductive events and body size. These results have implications in the design of laboratory experiments because it is a common practice to sample fish from a tank irrespective of social interactions that could be affecting critical parameters such as somatic growth or reproductive performance.

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Leptosynapta inhaerens (O.F. Müller 1776) (Echinodermata, Holothuroidea): A new record for the Belgian marine waters

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ABSTRACT. This is the first record of *Leptosynapta inhaerens* (O.F. Müller, 1776) in Belgian marine waters and adds a second apodid species, the first being *Leptosynapta minuta* (Becher, 1906), to the Belgian holothuroid fauna. This paper contains a morphological description of the specimens, the habitat characteristics, and includes a world distribution map of *L. inhaerens*. Relationships between *L. inhaerens* and environmental data are discussed.

KEY WORDS: Synaptidae, *Leptosynapta,* North Sea, new occurrence, distribution

INTRODUCTION

Belgian marine waters are, to date, known to harbour only six holothuroids: five Dendrochirotida: *Aslia lefevrei* (Barrois, 1882), *Neopentadactyla mixta* (Östergren, 1898) Deichmann, 1944, *Ocnus lacteus* (Forbes & Goodsir, 1839), *Ocnus planci* (Brandt, 1835) & *Thyone fusus* (O.F. Müller, 1776) (Massin, 1988; Massin & Deridder, 1989; Vandepitte, 2010) and one Apodida: *Leptosynapta minuta* (Becher, 1906) (Massin et al., 2005). The current species was collected with a Van Veen grab by the Institute for Agricultural and Fisheries Research (ILVO) on sandy muddy bottoms close to the Buiten Ratel sandbank. Careful inspection of the specimen revealed that it represents a sixth holothuroid species in Belgian marine waters. This paper describes *Leptosynapta inhaerens* (O.F. Müller, 1776) as a new record of an apodid holothuroid species in Belgian marine waters.

MATERIALS AND METHODS

The sampling station (BRN10 – 2012/194/24702), where the first *L. inhaerens* was found, is situated in the northern part of the Buiten Ratel sandbank (coordinates: 51°18.79 Lat – 2°36.52 Long (WGS84)), in a zone where aggregate extraction takes place (Map 1: C1). The sample was taken on the 3rd of October 2012 during an ILVO sampling campaign on board the oceanographic research vessel A962 Belgica. Using a Van Veen grab (sampling surface area: 0.1 m²), a sediment sample was taken and rinsed through a 1mm mesh sieve, after which the animals remaining were fixed with an 8% buffered formaldehyde-seawater solution. To determine the grain size distribution, a subsample of the sediment sample (2012/194/24765) was taken with a 3.6 cm Ø core prior to sieving. This sample was sieved in the laboratory through a 1.6 mm sieve to calculate the coarser sediment proportion. The rest of the sample was passed through a Malvern Mastersizer, to determine the grain size distribution of the sand/silt fraction by means of laser diffraction.

The first specimen found was broken into two pieces. It was stained with eosin to facilitate sorting and preserved in alcohol (90%) before transfer to the Royal Belgian Institute of Nature Sciences (RBINS). There the specimen was stored in buffered alcohol (70%, pH 8.3) and registered under N° IG 32529/1.

Recently, a second specimen (also in two pieces) was collected in sediment samples taken with a Hamon grab during a RBINS Belgica campaign (pers. comm. F. Kerckhof). The samples were taken on the 3rd of July 2013 in the Westhinder area just west to the Oosthinder sand bank (coordinates: 51°24.75 Lat -2°31.61 Long (WGS84)) in between barchan dunes (Map 1: C2). It is registered under N° IG 32529/2 in the collections of the RBINS.

Tissue from the body wall, longitudinal muscles and one tentacle were prepared for detailed examination. To preserve the integrity of the specimen as much as possible, only 2 of the 12 tentacles were removed for preparation. For the same reason no dissection has been performed to observe the internal anatomy. The ossicles were mounted on slides according to the method of SAMYN et al. (2006).

DESCRIPTION

Order Apodida (Brandt, 1835)
Family Synaptidae (Burmeister, 1837)
Subfamily Leptosynaptinae (Smirnov, 1989)
Genus *Leptosynapta* (Verrill, 1867)

Leptosynapta inhaerens (O.F. Müller, 1776)
Figs 1A-B, 2A-E; Maps 1-2

Holothuria inhaerens (O.F. MÜLLER, 1776): 232; 1781: pl.31, figs 1-7.
Synapta inhaerens (von DÜBEN & KOREN, 1844): 322: tab V, figs 56-62.
Leptosynapta inhaerens (VERRILL, 1867): 325.

(SOOK SHIN, 2012): 17, figs 7, 8A-E gave a long list of citations and synonymy for *Leptosynapta inhaerens*. To this list we can add: (KOEHLER, 1927): 270, pl XV, Fig 21; (TORTONESE, 1965): 104, Fig. 45; (PÉREZ-RUZAFA & LOPEZ-IBOR, 1988): 357; (HAYWARD & RYLAND, 1990) vol. 2: 871, Fig. 15-14; (PÉREZ-RUZAFA et al., 1992): 176, Fig. 2F; (MADSEN & HANSEN, 1994): 102, Figs 3B, C, 64A, 65-66, map 26; (MCKENZIE, 1997): 275; (PICTON, 1993): 83, Fig. b; (HANSSON, 2001): 350; (SOUTHWARD & CAMPBELL, 2006): 228, Figs 211 A-F.

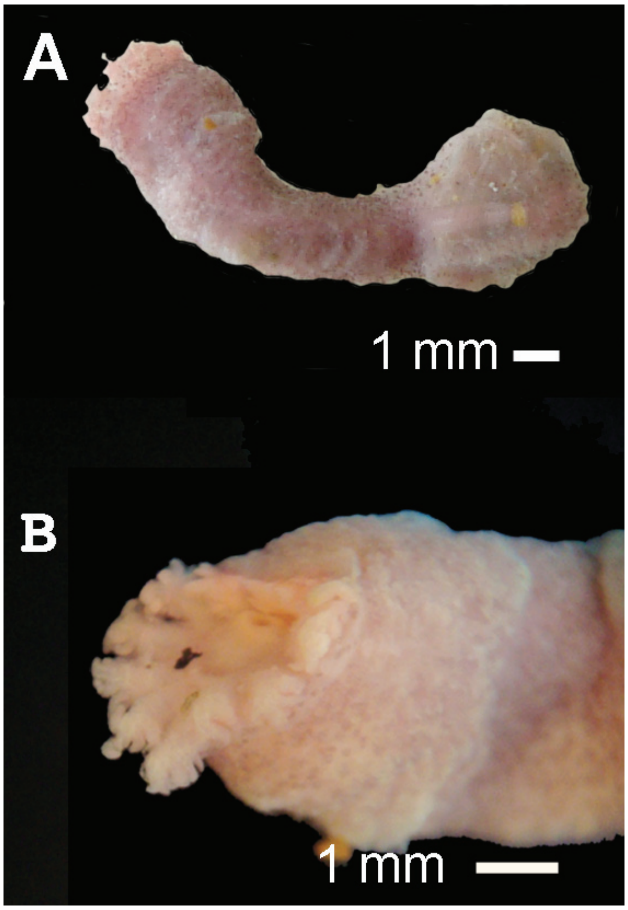


Fig. 1. – *Leptosynapta inhaerens* (O.F. Müller, 1776). **A**. Anterior body part. **B**. Tentacle crown.

Both specimens appeared to be broken in two. The anterior part of the first specimen, containing the tentacles, was 10.4 mm long and 3 mm across (Fig. 1A). The posterior part was contorted, the length estimated at ±7 mm. The posterior part had the same diameter as the anterior part. The specimen had twelve tentacles, which were short with three pairs of digits per tentacle. The terminal digit was single and larger than the laterals (Figs 1B, 2A). The body wall was covered with numerous reddish-brown dots. To preserve integrity of the specimen, no dissection was performed. Consequently, no information on internal anatomy (e.g., calcareous ring) is available.

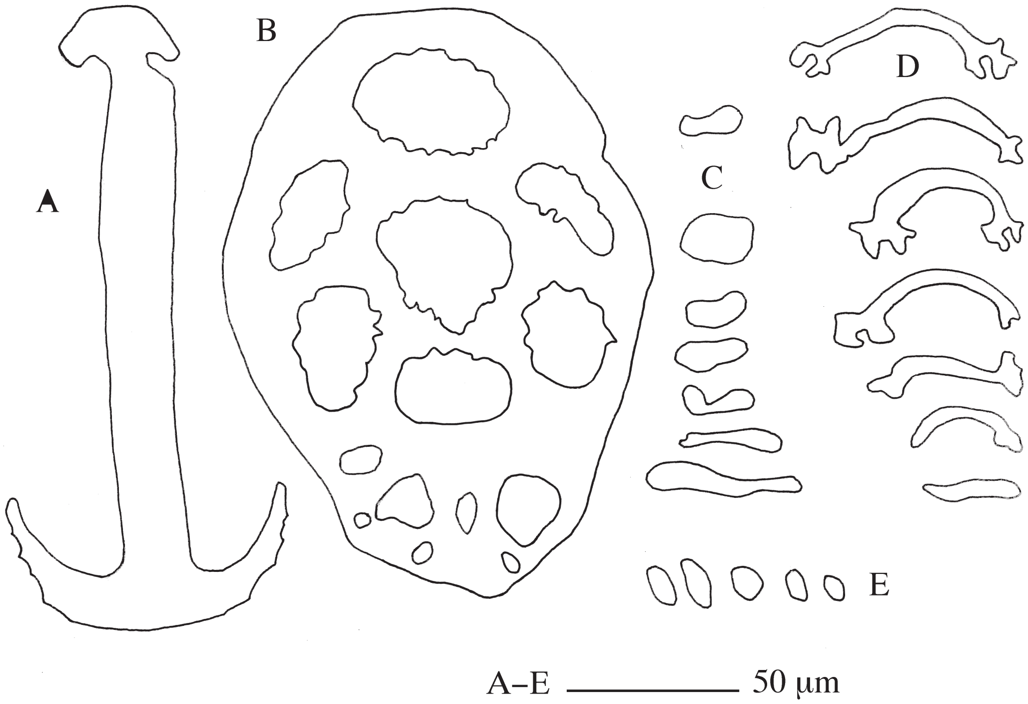


Fig. 2. – *Leptosynapta inhaerens* (O.F. Müller, 1776). **A**. Anchor. **B**. Anchor plate. **C**. Grains of longitudinal muscles. **D**. Tentacle rods. **E**. Tentacle grains.

The ossicles of the body wall comprised anchors and anchor plates. Anchors were 180-220 µm long and 80-100 µm wide with 3-4 teeth on arms and a smooth apex (Fig. 2A). Anchor plates were 130-160 µm long and 100-170 µm wide. The edge of the anchor plates was smooth; the anterior side perforated by seven dentate holes. The posterior side was perforated by 5-8 smooth holes (Fig. 2B). Ossicle variations (mainly the number of holes in the posterior part of the anchor plates) occur within one specimen.

The specimen had longitudinal muscles with grains, 25-50 µm long (Fig. 2C). The tentacles had small smooth curved rods 30-70 µm long (Fig. 2D) and a few grains 15-20 µm long (Fig. 2E).

The second specimen measured 50 mm long and 2 mm across (pers. comm. F. KERCKHOF). The identification was based on examination of the anchors and anchor plates.

Leptosynapta inhaerens is a highly variable species (e.g., number of digits (3-10 pairs) per tentacle, number of sense cups (2-14) per tentacle, number of holes (6-14) in the posterior part of anchor plates); already 12 synonyms were mentioned by CLARK (1907). Ossicles vary according to the size of the specimen and to the locality (CLARK 1907: 88-89; MADSEN & HANSEN, 1994: fig. 66). As a consequence, this species is often confused with *Leptosynapta bergensis* (Oestergren, 1905) and *Leptosynapta tenuis* (Ayres, 1851) (MADSEN & HANSEN, 1994; SOUTHWARD & CAMPBELL, 2006).

Using the following dichotomous key, separation can be made between the above described species (for illustrations of O-ring ossicles see HEDING (1928): figs 22-40).

Key to the species *L. minuta*, *L. inhaerens*, *L. tenuis* and *L. bergensis*

1. Ten tentacles without digits ....*L. minuta*
– Twelve tentacles with digits 2

2. O-ring ossicles absent in longitudinal muscles ....*L. inhaerens*

– O-ring ossicles present in the longitudinal muscles ……………………………………3

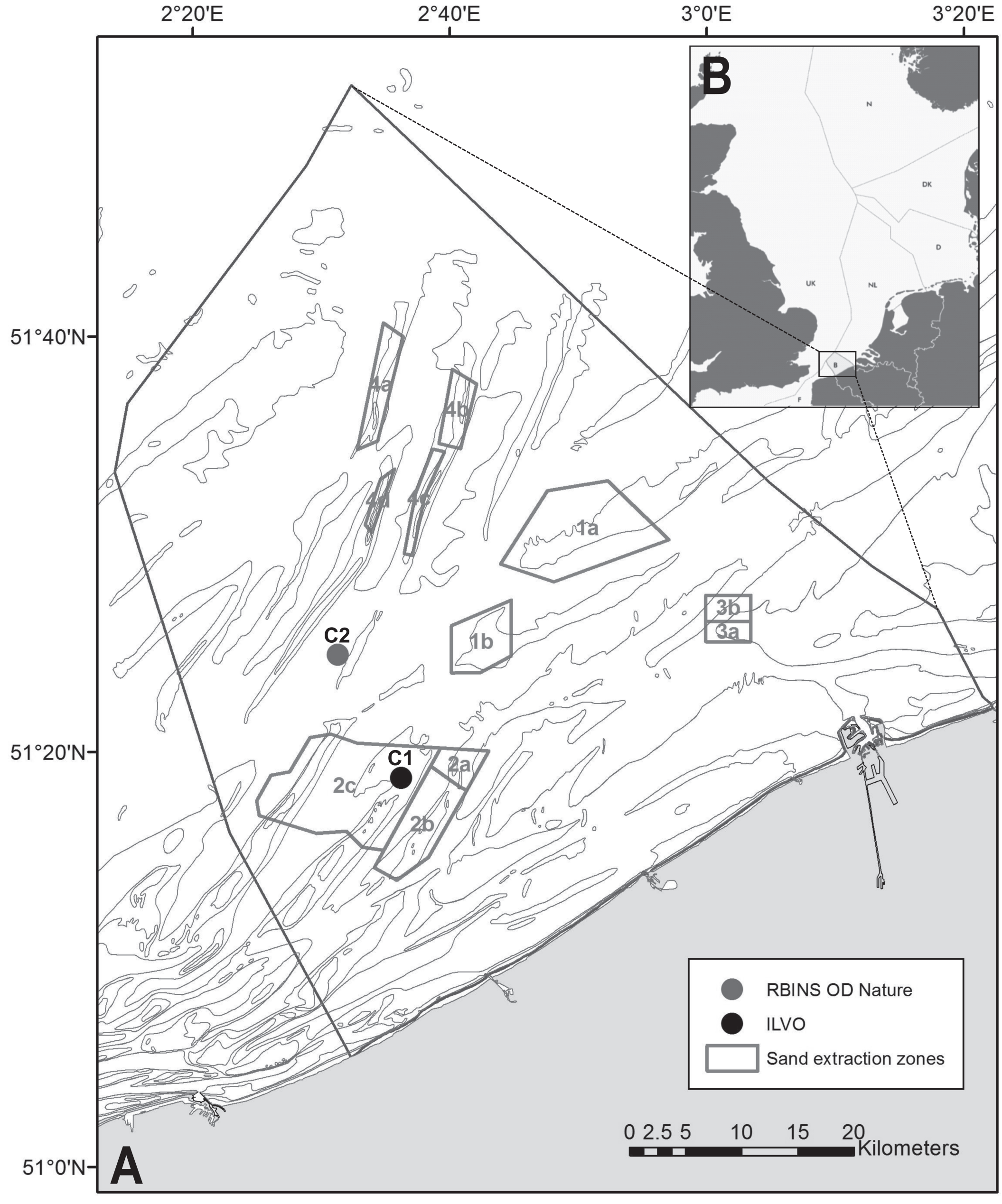
3. Rods of tentacles around 30 µm long with end faintly enlarged ……………………*L. tenuis*

– Rods of tentacles around 45 µm long with enlarged and branched ends ……*L. bergensis*

HABITAT CHARACTERISTICS

Sample depth from the Buiten Ratel ranged between 15 and 20 meters. Water temperature and salinity were 15.9 °C and 34.7 (PSU), respectively. The sediment could be characterised as medium sand, with a median grain size of 265 µm. The dominant sand fraction (48%) was medium sand (250-500 µm), followed by the fine sand fraction (125-250 µm) (41%). The coarse fraction (>1600 µm) was limited to 7% of the total mass.

The sampling area was situated in a sand and gravel exploitation zone (Zone 2c) (see Map 1). Intensive extraction activities since 2006 have



Map 1. – Location of the VAN VEEN grab sample. **A**. Belgian waters. **C1**. BRN 10 (located in a zone of high aggregate extraction activities). **C2**. Location of the Hamon grab sample.

resulted in the formation of a depression near the sampling area (De Backer et al., 2011).

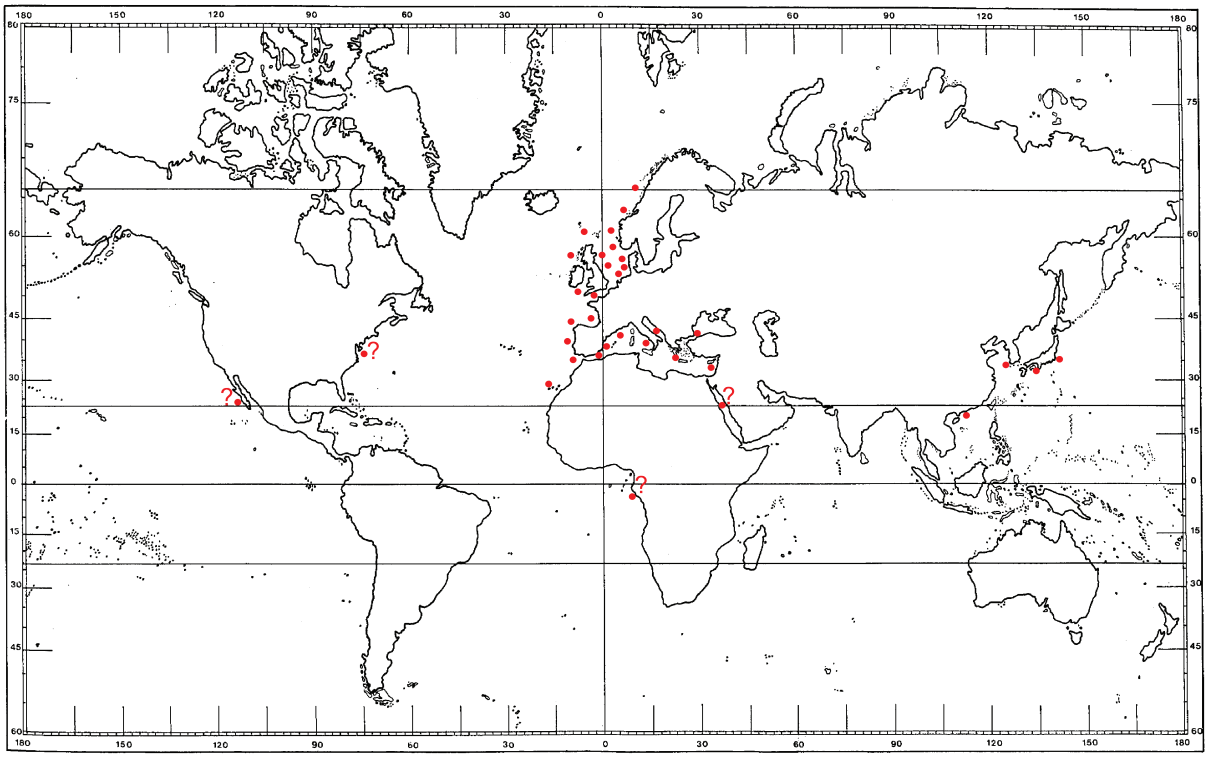
The second specimen was encountered in a mixed sediment type (muddy sand containing a considerable amount of shell debris, and also small cobbles and stones (pers. comm. F. Kerckhof).

DISTRIBUTION

Type locality: Southern Norway. *Leptosynapta inhaerens* is a widespread species mentioned in numerous publications. It has been recorded from the North Sea to northern Norway, all around England, the Atlantic coast of Denmark, Germany, France, Spain, Portugal, the Canary Islands, the Mediterranean coast of Spain, France, Italy, the Adriatic Sea (Greece), the Black Sea, Israel, Korea, Japan and China (see Map 2). This map is mainly based on the work of Koehler (1927), Pérez-Ruzafa & Lopez-Ibor (1988), Madsen & Hansen (1994) and Sook Shin (2012). However, the presence of *L. inhaerens* in Korea, Japan and China (Sook Shin, 2012) is still uncertain, because all the specimens studied had O-ring ossicles in the longitudinal muscles (Oguro, 1961; Yi, 1985; Sook Shin, 2012). These ossicles appear to be absent in *L. inhaerens* from the North Atlantic and Mediterranean Sea (Clark, 1907; Bush, 1918; Heding, 1928; Hayward & Ryland, 1990; Pérez-Ruzafa et al., 1992; Madsen & Hansen, 1994; Southward & Campbell, 2006). Based on these differences it is likely that the so-called *L. inhaerens* specimens from the Asiatic populations are in fact different species.

L. inhaerens has also been mentioned from Massachusetts (Clark, 1907), but according to Mortensen (1927) and Heding (1928) the American specimens are a different species. *Leptosynapta inhaerens* was also mentioned as being present in the Red Sea (Kosseir, Egypt) and the Congo (Lampert, 1885), but these records are not fully confirmed yet (Samyn, 2003).

Considering the above it is probably unwise to regard *L. inhaerens* as a cosmopolitan



Map 2. – World distribution map of *Leptosynapta inhaerens*. Dots with a question mark are doubtful.

species. However there is sufficient evidence confirming its presence in the North Atlantic and Mediterranean Sea.

DISCUSSION

For Belgian waters, *L. inhaerens* is newly recorded and is the second known apodid species in this area. These recent observations may even suggest a possible connection between the existing populations of the English Channel and the Southern Bight of the North Sea. Adults leptosynaptids are usually considered to be exclusively bottom-dwelling forms, but COSTELLO (1946) observed young ones (sizes from 30 to 50mm) to be floating near the surface of the water. In case of disturbance they stop their swimming movements, contract their bodies and sink slowly toward the bottom.

ILVO and UG researchers VANDEPITTE et al. (2010), CATTRIJSSE & VINCX (2001) and DEGRAER et al. (2008) have collected numerous Van Veen samples in the vicinity of, and on to the Buiten Ratel sandbank since 1970. Up till now this is the first registered specimen of *L. inhaerens*. The southern North Sea, with its sandy bottoms and shallow waters (0-50 m), is expected to be favourable to *L. inhaerens* (KOEHLER, 1927; MORTENSEN, 1927; MADSEN & HANSEN, 1994). However, according to MADSEN & HANSEN (1994) and MCKENZIE (1997) the species is supposed to be absent from the southern North Sea, whereas it appears to be an abundant species along the British Isles (MORTENSEN, 1927; SOUTHWARD & CAMPBELL, 2006). The unexpected rarity of *L. inhaerens* in Belgian marine waters could be related to the grain size distribution and aggregate extraction. Aggregate extraction can disturb the U-shape burrow in which the animal lives. Since 2006, considerable quantities of sand have been systematically extracted from the Buiten Ratel sandbank area (DE BACKER et al., 2011). However, these authors also noted a higher benthic diversity within the Buiten Ratel impact zone, and related this to an overall change in the physical environment (i.e., changes in the sandbank morphology) and the presence of finer sediments, which attracted several new species to the area. Similarly, the disturbed sediments probably also attracted this newly recorded apodid holothuroid species. The recent encounter of another apodid species in the Hinder sandbank area proves that *L. inhaerens* is clearly present in the Belgian marine waters. It remains unclear why the species was never found in the past. Is it because it always appears in very low abundances, minimising a possible catch? Or could its appearance be attributed to a lack of appropriate sampling (in other words, is the Van Veen grab the best collecting gear for small, fragile sea cucumbers)? Maybe future monitoring of both areas could answer these questions?

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SHORT NOTE

First record of Montagu's sea snail *Liparis montagui* (Donovan, 1804) in Belgian waters

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KEY WORDS: sea snail, *Liparis montagui*, Belgian part of the North Sea, new observation

This paper presents the first record of a fish species new to the Belgian Part of the North Sea, including its taxonomy and ecology, and discusses the occurrence of the species in the Belgian and neighbouring waters.

Montagu's sea snail *Liparis montagui* (Donovan, 1804) is a demersal fish species from the Liparidae family, occurring in the inshore waters of the North East Atlantic. It is found around southern Iceland and from the Barents Sea southwards through the North Sea and the Baltic Sea and along European coasts. Young specimens are also found in the Wadden Sea (1, 2, 3, 4). *L. montagui* occurs from the intertidal to 30 m, under stones at low tide, in rock pools or clinging to algae (e.g., *Fucus*). It feeds primarily on gammarid amphipods in intertidal areas and shrimps and small crabs in subtidal areas (2, 3, 5, 6).

A specimen of *L. montagui* was found on March 9th, 2011 at sampling location ft330 (N51.433, E2.815 - N51.427, E2.794) on the Gootebank at a depth of 23 m (Fig. 1). The sample was taken with an 8 meter-shrimp beam trawl (stretched mesh width 22 mm in the cod end) dragged over the bottom for 15 minutes at an average speed of 4 knots. The 40 l catch contained some larger stones, and dab *Limanda limanda* and whiting *Merlangius merlangus* as the most abundant fish species.

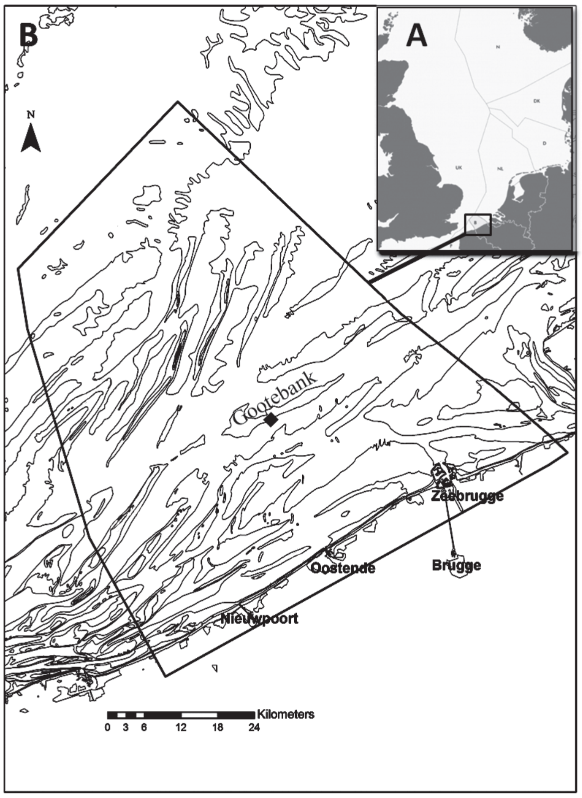

Fig. 1. – **(A)** North Sea exclusive economic zones. **(B)** Belgian part of the North Sea (BPNS) with sampling station ft330 where *Liparis montagui* was caught.

Table 1

Overview of literature concerning the number of dorsal and anal fin rays in *Liparis montagui* and *Liparis liparis*.

		NIJSSEN & DE GROOT[5]	**EALES & KEMP[6]**	**QUÉRO *et al.*[8]**	**HAYWARD & RYLAND[9]**	**LYTHGOE & LYTHGOE[10]**
Liparis montagui	**dorsal fin rays**	26-32	26-30	26-32	28-30	28-30
	anal fin rays	22-25	24	22-26	22-25	22-25
Liparis liparis	**dorsal fin rays**	32-36	34-36	32-45	33-35	33-36
	anal fin rays	26-30	27-28	26-38	27-29	27-29

Montagu's sea snail *Liparis montagui* is very similar in appearance and habits to the sea snail *L. liparis*. The pelvic fins of both species are modified into a suction disc on the belly and both are variably coloured. Still, a number of characteristics make it possible to distinguish the two species. *L. montagui* is purplish brown in appearance to the naked eye, but with the assistance of a lens one can see that the ground-colour is yellowish brown with dark spots. *L. liparis* on the other hand tends to be pale grey or brown, with darker brown or purplish longitudinal bands (6, 7). *L. montagui* can further be distinguished from *L. liparis* by its smaller size (max. 12 cm *versus* 18 cm), the never overlapping dorsal and caudal fins, the rarely overlapping anal and caudal fins, the smaller number of rays in the dorsal (26-32 *versus* 32-45) and anal fins (22-26 *versus* 26-38) and the posterior nostrils in adults that are covered by skin (3, 5, 6, 8, 9, 10).

Since fin ray counts provide the most reliable distinction between the two species (11), we particularly focused on this characteristic. Table 1 gives an overview of the literature concerning the number of dorsal and anal fin rays in both species. Table 2 presents the identification characteristics of the found specimen and a *L. liparis* specimen caught at another location in the Belgian part of the North Sea, compared to the minima and maxima found in literature. The photograph (Fig. 2) and drawing (Fig. 3) of the found specimen clearly show that neither the dorsal nor the anal fins overlap with the caudal. Also, the fin ray counts of the anal and dorsal fins of the found specimen clearly correspond with the characteristics of *L. montagui*. We can conclude that this is the first unequivocal evidence of a recording of *L. montagui* in the Belgian part of the North Sea.

Table 2

Examined identification characteristics compared with literature.

	Literature (*min-max values taken from table 1)					**Own specimen**		
	dorsal/ caudal overlap[3]	**anal/ caudal overlap[3]**	**maximum length[19]**	**dorsal fin rays***	**anal fin rays***	**dorsal fin rays**	**anal fin rays**	**fin overlap**
Liparis montagui	no	rarely	120 mm	26-32	22-26	**29**	**23**	**no**
Liparis liparis	yes	yes	180 mm	32-45	26-38	**33**	**28**	**yes**

Fig. 2. – Photograph of the fresh *Liparis montagui* specimen caught in BPNS (© Hans Hillewaert).

During our long-term monitoring programs, regular beam trawl samples have been taken for over 20 years on the Belgian part of the North Sea. These data showed that *L. liparis* is a common species throughout the area (4) while *L. montagui* was never observed (pers. obs.). There was uncertainty about the possible observation of *L. montagui* during a sampling campaign on September 24th, 2007 (4 years prior to the reported observation), but unfortunately due to the inferior condition of the specimen we were unable to make a straightforward identification. Hence, the recording of March 9th, 2011 is considered to be the first official reported catch of *L. montagui* for the Belgian marine waters. Since then, *L. montagui* has been reported from Belgian waters by Hans De Blauwe (12) at the marina of Zeebrugge and by Kelle Moreau (pers. comm.) at the coastal zone of Nieuwpoort, the latter caught on September 9th, 2013 from R.V. Simon Stevin with a 6 meter shrimp beam trawl and a similar small mesh net. In both cases, the specimen was not preserved and the identification could not be confirmed. However, these observations may indicate that *L. montagui* has established a resident population along the Belgian coast. *L. montagui* has been reliably reported in the UK (13). Also WHEELER (14) has examined specimens from various parts of the coasts of England, Wales and the Isle of Man. In the North of France (Nord-Pas-de Calais), the species was inventoried by Glaçon in the 1970s, and it was identified in the updated inventory of MÜLLER (2004) as a common species for this region (15, 16). In the Netherlands, *L. montagui* is considered a very rare species and its protection status is covered by national and international legislation (17, 18).

We can assume that *L. montagui* has been present in the Belgian part of the North Sea for some time already, but probably in very low

Fig. 3. – Drawing of the dorsal and caudal fin rays of the same fixed specimen of *Liparis montagui* (© Hans Hillewaert).

numbers. The small population, small size and limited distribution make it difficult to capture representatives of this species (13). The fact that our monitoring program normally covers soft sandy sediments, and *L. montagui* is a typical species of rocky sediments, contributes to this. Although location ft330 has always been a 'stony' habitat, *L. montagui* was not previously caught in this area. Perhaps climate change has favoured the appearance of this species in Belgian waters in recent years. Also, the recent introduction of wind farms (and hard substrates) in the vicinity of this area may act as an attraction pole for *L. montagui*.

Increased monitoring efforts will probably reveal more species that are new to the Belgian fauna in the future. For now, we have shown that Montagu's sea snail *L. montagui* is indeed present in the Belgian part of the North Sea.

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