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THESIS

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SÉPARATION DE NICHE ENTRE DEUX ESPÉCES INVASIVES DE GOBIES

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Faithfully yours

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Résumé

Comprendre la coexistence d'espèces et le maintien de la biodiversitéest depuis longtemps un point central en écologie des communautés. La notion de niche, ou théorie des niches, a été largement développée depuis l’explication par Darwin de l’impressionnante diversité de la vie sur Terre. Celle-ci est considérée comme un mécanisme majeur permettant la coexistence d’espèces compétitrice au sein des communautés écologiques. La différenciation en niches spécifiques implique des différences spatiales, trophiques, temporelles et/ou une combinaison de celles-ci. Dans cette étude, la sélection d’habitat, les traits d’histoire de vie, la composition alimentaire et les comportements alimentaires de deux espèces invasives de gobiidés, très abondantes et écologiquement similaires (*Rhinogobius cliffordpopei* et *Rhinogobius giurinus*), sont comparées afin de tester si la séparation de niches est un mécanisme qui peut potentiellement permettre leur coexistence stable dans le lac Erhai (un lac superficiel d’eau douce du plateau de Yunnan-Guizhou en Chine).

Les résultats montrent qu’il y a séparation des niches de ces deux espèces le long d’un axe d’habitat, d’un axe alimentaire (pour l’habitat littoral et pour les adultes et sub-adultes), et d’un axe temporel (en termes de cycles de ponte).

Spécifiquement, *R. giurinus* occupe principalement les habitats profonds (PH) alors que *R. cliffordpopei* occupe principalement l’habitat littoral (LH). Des analyses corrélatives ont montré que l’abondance de *R. giurinus* est positivement associée avec la profondeur de l’eau et les substrats composés de sables limoneux et grossiers, tandis que la distribution de *R. cliffordpopei* est corrélée aux fortes densités en macrozooplankton, les fortes abondances en autres espèces de poissons, les fortes concentrations en oxygène dissout et les fortes densités en macrophytes submergées.

Concernant le partage en ressources alimentaires, les deux espèces ont montré de clairs changements au niveau de leur diète ontogénique ainsi que dans les patrons de partage des aliments qui sont significativement différents selon le stade de leur histoire de vie et les habitats.

Pour les deux espèces, les juvéniles consomment principalement du macrozooplancton (cladocères et copépodes), alors que les sub-adultes et les adultes consomment principalement des larves de Chironomidae et de Tubificidae. En LH, les sub-adultes et adultes des deux espèces présentent des différences significatives dans leur régime alimentaire alors que les juvéniles ont des régimes similaires. Cependant, en PH et SH, nous n'avons trouvé aucune preuve de partage des ressources alimentaires, quelque soit le stade de vie (juvéniles, sub-adultes et adultes).

R. cliffordpopei et *R. giurinus* présentent un partage de leur niche temporel. En effet, les deux espèces ont des débuts de période de reproduction ainsi que des pics de reproduction à des moments différents. *R. cliffordpopei* se reproduit de Février à Juin avec un pic de ponte entre Mars et Avril alors que l'activité de reproduction de *R. giurinus* se fait entre Avril et Août avec un pic de ponte pendant les mois de Mai et Juin. Ces différences ont conduit à un partage temporel des cohortes de taille similaire entre les deux espèces, en particulier pour les larves de petite taille et les juvéniles, qui sont presque exclusivement zooplanctivores et qui exploitent les mêmes ressources alimentaires dans le lac. Cependant, l'activité nycthémérale (activité de locomotion) et le rythme d'alimentation varient légèrement entre les deux espèces, i.e. les deux espèces présentent deux pics d'activités (6:00 - 10:00 et 18:00 - 22:00) et deux principales périodes d'alimentation (6:00 - 10:00 et 18:00 - 22:00) sur un laps de temps de 24h pour les quatre saisons.

De plus, notre étude apporte une compréhension complète de la sélection d'habitat et des traits d'histoire de vie (i.e. patron de croissance, biologie de la reproduction et dynamique de population) de ces deux espèces invasives de gobies. Ces résultats biologiques sont essentiels pour la mise en place de programmes économiquement et écologiquement efficaces de contrôle pour les deux espèces de ravageurs. De nouveau programmes de gestion sont fortement recommandés en vue de ces résultats. Par conséquent, dans cette étude, plusieurs programmes de restauration spécifiques et innovants sont proposés pour améliorer les stratégies

actuelles de gestion dans une perspective de réduction des coûts et une meilleure efficacité.

Mots clés: coexistence d'espèces, espèces de gobies, théorie des niche, séparation de niche, invasion écologique, gestion d'espèces invasives.

Abstract

Understanding species coexistence and maintenance of biodiversity in nature has long been a central issue in community ecology. The niche or niche-based theory has been developed since Darwin's explanation of an amazing diversity of life on the Earth and considered as a major theory explaining the coexistence among competing species within ecological communities. Niche differentiation basically involves spatial, trophic, temporal and/or a combination of them. In the present study, habitat selections, life characteristics, diet compositions and feeding behaviors of two highly abundant and ecologically similar invasive goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) are compared to test whether niche separation is a potential mechanism allow ing the stable coexistence of them in Lake Erhai (a shallow freshwater lake in the Yunnan-Guizhou Plateau of China).

The results demonstrated that these two species showed niche separation along habitat axis, food axis (in littoral habitat for sub-adults and adults), and temporal axis in terms of spawning cycles.

Specifically, *R. giurinus* mostly occupied profundal habitat (PH) while *R. cliffordpopei* mainly used littoral habitat (LH). Correlation analyses revealed that the abundance of *R. giurinus* was positively associated with deep water, silt and coarse sand substrata, whereas the distribution of *R. cliffordpopei* was positively associated with high densities of macrozooplanktons and high abundances of other fish species, high concentration of dissolved oxygen and high densities of submerged macrophytes.

For food resources partitioning, they showed a clear ontogenetic diet shifts and significantly different food partitioning patterns at different life stages and habitats. For both species, macrozooplanktons (Cladocera and Copepoda) were the main food for juveniles while sub-adults and adults primarily consumed Chironomidae larvae and Tubificidae. In LH, sub-adults and adults of the two species significantly differed in their diet compositions while juveniles showed similar diets. In PH and

SH, however, there was no evidence for food partitioning between species at all the three life stages.

R. cliffordpopei and *R. giurinus* showed temporal niche partitioning as they exhibited different onsets of spawning and peaks of spawning seasons, and thus different growth and population dynamics, though both of them are short-lived species with a life span of about one year. *R. cliffordpopei* spawned from February to June with a spawning peak occurring in March and April. Spawning activity of *R. giurinus* occurred from April to August with a peak activity during May and June. These differences resulted in a temporal partitioning for similar-sized cohorts, especially for small-sized larvae and juveniles, which were almost exclusively zooplanktivorous and exploited the same food resources in the lake. Moreover, their different spawning cycles led to different peak seasons for the highest population density of the two species. However, the diel activity (locomotory activity) and feeding rhythm varied slightly between them, i.e. both species showed two peaks of activity (6:00 - 10:00 and 18:00 - 22:00) and two main feeding periods (6:00 - 10:00 and 18:00 - 22:00) in the four seasons.

Moreover, our study provides a full understanding of habitat selection and life characteristics (i.e. growth pattern, reproduction biology and population dynamic) of the two invasive gobies. These findings are crucial biological aspects for an economically and ecologically effective control programs to the two abundant pest species. The further management programs are highly recommended to give a careful consideration of these findings. Therefore, several specific remediation is suggested to improve the current management strategies from the perspective of cost-efficiency.

Key words: species coexistence, goby species, niche theory, niche separation, biological invasion, invasive species management

Part I: Synthesis

Introduction

1.1 Mechanism of species coexistence

A central question in community ecology is how to synthetically explain the observed patterns of species abundance in space and time, and across scales (Chesson, 2000; Hubbell, 2001; Amarasekare, 2003; Clark, 2009). The theoretical principle of competitive exclusion, also called Gause's law (Gause, 1934), predicts that one species would eventually outcompete and displace the other when they compete for the same critical resources within an environment. However, a striking pattern in nature is not to be the case in a large number of ecologically similar species. For instance, plant alpha diversity reaches astonishing levels in tropical forests (e.g. a single hectare of Amazonian forest can support more than 280 tree species Wright, (2002)). To obtain synthetic explanations for stable coexistence of species, several theories and hypotheses have been put forward, of which the two most influential ones are the “niche or niche-based theory” (Chesson 2000; Chase & Leibold, 2003; Silvertown, 2004) and the “unified neural theory” (Hubbell, 2001; Chave, 2004; Hubbell, 2005; Hubbell, 2006). These two concepts have sparked considerable controversy over more than two decades among ecologists (Gaston & Chown, 2005; Gewin, 2006; Adler *et al*., 2007; Clark, 2009).

The “niche or niche-based theory” (e.g. Lotka-Volterra competition model (Leigh, 1968); regeneration niche hypothesis (Grubb, 1977); storage effect (Caceres *et al*., 1997); resource ratio hypothesis (Tilman, 1982); microbial mediation hypothesis (Zobel *et al*., 1997); competition–colonization trade-off (Levine & Rees, 2002)) has dominated community ecology for nearly a century (Leibold, 1995; Chesson, 2000; Chase & Leibold, 2003). This theory assumes that every organism has a place or position to live in natural environment, in which there is a functional role of the living organism and a complex set of adaptations for propagating their offspring (Grinnell, 1917; Rejmanek & Jenik, 1975). The ecological niche is a term describing the way of life of a species, i.e. how an organism and/or population responds to various abiotic and biotic factors (e.g. resources, competitors, predators, parasites, pathogens, etc.) and how it, in turn, alters those factors (e.g. acting as a food source for predators and a consumer of prey, limiting access to resources by other organisms, etc. (Rejmanek & Jenik, 1975; Leibold, 1995)). Since many environmental factors may influence the living of a species in nature, Hutchinson (1957) took niche definition one step further by a quantitative formulation giving a full consideration of all the environmental factors. He viewed the fundamental niche of a species as an *n*-dimensional hyper-volume, in which the dimensions are environmental conditions and resources that define the requirements of an individual or a species (Hutchinson, 1957). Each of these different dimensions (i.e. a plot axis) of a niche represents a biotic or abiotic variable in the environment. Hutchinson's "niche" is the ecological space occupied by a species and also incorporates ecological "role" of the organisms, which may provides an actual snapshot of the “living” of a species in nature (Rejmanek & Jenik, 1975; Leibold, 1995; Kylafis & Loreau, 2011).

The niche concept was popularized by Hutchinson but ecologists have long intrigued why there are so many different types of organisms within a habitat as competitive exclusion principle (Gause, 1934) predicts that two species can mere occupy the same niche in the same environment for a long temporal (Chesson 2000; Silvertown, 2004). Thus, each species is thought to have a separate, unique niche across spatial and/or temporal scales, and the niche of a single species is the multivariate combination of environmental features within a particular set of abiotic and biotic conditions (Jones *et al.*, 2001; Wright, 2002; Chase & Leibold, 2003). Subsequently, niche differentiation among species is suggested as a major mechanism of coexistence of species in ecological communities (Schoener, 1974; Ross 1986; Jones *et al.*, 2001; Amarasekare, 2003; Kylafis & Loreau, 2011). The classic niche concept is a fundamental and central aspect of modern ecology, yet the understanding of the mechanisms of species coexistence has remained unclear by the niche only. The assumption of the niche of a species might remain unchanged, or change only slowly over hundreds to millions of years (Wiens & Graham, 2005; Pearman *et al*., 2008).

Over the past two decades, Hubbell (2001) has developed the "unified neutral theory of biodiversity and biogeography" that has become a dominant part of biodiversity science and one of the concepts most often tested with field data and evaluated with models (Adler *et al*., 2007; Clark, 2009). This theory directly challenges the niche paradigm as it assumes that species are ecologically similar and equivalent with regards to the probabilities of birth, death, dispersal and speciation (Gaston & Chown, 2005; Gewin, 2006; Clark, 2009). It raises an extreme and provocative assumption that all individuals are ecologically identical, largely suggesting that niche differences are not needed to explain biodiversity patterns (Bell, 2001; Hubbell, 2005; Hubbell, 2006).

The neutrality originates from island biogeography and neutral theory of molecular evolution (random genetic drift). First, neutral assumption is that differences among species are proposed to have no substantial effect on biodiversity within a community, though individuals of certain species usually show some characteristics that make them look or function differently with other species (Bell, 2001; Hubbell, 2001). Second, the community is saturated, in which individuals are engaged in a zero-sum game whereby if one emigrates or dies another one will immigrate or be born to take its place. However, there is no influence of individual's traits (e.g. life characteristics) over the contribution to community saturation because all important ecological aspects associated with those species are equivalent (Gaston & Chown, 2005). Random death, dispersal and speciation are the most important features in the neutral theory (Hubbell, 2001). Neutral theory predicts the possibility of highly diverse communities of equivalent species due to the assumption of fitness equivalence, which is, in fact, a stochastic or random process including birth, death, immigration, emigration and speciation. Thus, it is often described as a "dispersal-assembly" theory or a "stochastic" theory (Bell, 2001; Hubbell, 2006). Since all individuals are functionally equivalent and processes are neutrally driven by random events, the coexistence of species is usually unstable in neutral communities (Chave *et al*., 2004).

Surprisingly, given its extreme simplification of a seemingly complex phenomena (i.e. species equivalence), the neutral theory has attracted enormous attention among ecologists and has successfully described the observed species–area relationships and species abundance distributions in several communities, especially in equatorial rainforests and coral reefs (Gewin, 2006). Those ecosystems are thought to be the best example of neutral dynamics because species are highly diverse with a limited potential to partition resources into niches. Niche theories, however, are hard to explain more generally in communities with many rare species and only a few abundant ones (Tilman, 2004; Gewin, 2006).

Over the last decades, the monograph *"The Unified Neutral Theory of Biodiversity and Biogeography"* (Hubbell, 2001) has provoked vigorous controversy among ecologists. The assumption that species are equivalent to each other in all important ecological aspects, which is far different from typical niche-based assumption that species are highly different from each other owing to the variations of niche requirements and "living" ability (e.g. ability for competition, exploitation resources and reproduction (Gewin, 2006; Leibold & McPeek, 2006; Clark, 2009)). The main disagreement of neutral theory is the extreme assumption of ecological or functional equivalence among all the species, which seems to completely ignore the differences in species-level traits such as habitat preferences, reproductive strategies physiological tolerances, dispersal abilities, etc. (Gaston & Chown, 2005). Another constraint of the neutral theory is that it only applies under some given circumstances and this makes it appropriate in such circumstances without a wider applicability. For instance, it primarily lies in attempts to explain the high diversity of tree species in tropical forests or fishes in coral reefs, and particularly concerns trophically similar groups (Hubbell, 2001; Chave, 2004; Gaston & Chown, 2005). On the other hand, despite the fact that the niche theory has been long developed and can explain many observed assemblage and community structure (Chesson, 2000;

Chase & Leibold, 2003; Silvertown, 2004), it ignores the neutral process in nature (Gaston & Chown, 2005; Leibold & McPeek, 2006). At the very beginning, ecologists often treat niche theory and neutral theory as mutually exclusive explanations, but they now recognize that the controversy is, at the most, over the relative importance of niches and neutrality or unifying niche and neutral theories (Gaston & Chown, 2005; Leibold & McPeek, 2006; Adler *et al*., 2007). It is indeed a false for a dichotomy between niche and neutrality obscuring a fact that there are two processes simultaneously influencing the community of competing species (Tilman, 2004; Leibold & McPeek, 2006; Adler *et al*., 2007). Ironically, niche and neutral theory have reinvigorated each other as Gewin (2006) stated "the prevailing notion is that stochastic forces exist on one end of a continuum while deterministic forces occupy the other. Finding any truth that lies between is the challenge. It's not niche or neutral…it's determining the relative importance of the two".

1.2 Niche theory

One of the most fundamental concept in modern community ecology is the ecological niche. Ecologists use niche concept to organize a general thought about the "living" ways of organisms in nature, in which organisms use resources, interact with each other, and assemble into communities or ecosystems (Leibold, 1995). The niche of a species is a result of evolutionary and natural selective processes, through which it fits itself into an ecological community or ecosystem by morphological, physiological, and behavioral adaptations (Tokeshi & Schmid, 2002; Nosil & Sandoval, 2008; Kylafis & Loreau, 2011). Therefore, ecological niche describes not only a physical position (i.e. habitat conditions necessary for persistence of the species) but also a functional role of a certain species in ecosystems. Niche is a very basic and fundamental ecological concept subsuming all of the interactions within a species and also the response to biotic and abiotic environments (Leibold, 1995).

The niche concept has been developed more than a century. The first attempt to describe the organism-environment relationships is the Darwin's phrase "place in natural economy" and Sencer's term "correspondence" (Whittaker *et al*., 1973; Rejmanek & Jenik, 1975). Darwin (1859) refers to species "filling nearly the same place in the natural economy of the land" and to "a place in the natural polity of the country". Darwin's idea is the essence of the later united term "niche" in Grinnell (1917), Elton (1927), Hutchinson (1957) as well as other proponents (Rejmanek & Jenik, 1975).

Grinnell is generally acknowledged to be the first ecologist developing the ecological concept of the niche, though he is the not the first one using the word of "niche" (Whittaker *et al*., 1973; Leibold, 1995). In the typical paper "*The niche-relationships of the California Thrasher*" (Grinnell, 1917), he demonstrated that temperature was the main constraint on geographical range of thrashers. Moreover, he found that the dense bush crown was particularly important for thrashers to avoid predators. Therefore, those factors were defined to organize the niche of thrashers since they seemed to explain the thrasher's distribution. Although Grinnell is interested in environmental factors (e.g. physical or climatic) that restrict a species' potential geographical distribution, he makes greatly contribution towards the later development of niche theory whereby two fundamental thoughts, i.e. species originally evolve to fill their niches and no two species could have exactly the same niche (Grinnell, 1917).

Compared with Grinnell's niche concept, the exciting progress by Charles Elton is related the niche of an organism to their food and enemies instead of the only sense of geographical distribution (Elton, 1927). Elton's definition of niche, in fact, referred to an environmental "place" as well as ecological or functional role in its community. Elton made a great stride by incorporating the "role" to niche concept (Whittaker *et al*., 1973). A few years later, a central tenet of modern niche theory, "Gause's principle" or "competitive exclusion principle", was published by Georgii Frantsevich Gause (1934).

The niche concept had been not developed greatly until the new definition by George Evelyn Hutchinson (1957), who gave a full consideration of all the environmental factors as well as functional roles of a species in nature, and viewed the fundamental niche of a species as an *n*-dimensional hyper-volume. It is the first concept with a rigorous and quantitative formula of niche theory rather than the nebulous ones prior to Hutchinson (Whittaker *et al*., 1973; Rejmanek & Jenik, 1975). This formalization directly enables the measurement of the niche of an organism and also comparison of niches between two or more species. Moreover, Hutchinson recognized the "fundamental niche", which is a full range of conditions (biotic and abiotic) and resources that could be used by a species completely free of any interference with other species under an ideal environment. The fundamental niche of a certain species is largely determined by heredity factors (e.g. life characteristics, feeding habits, morphological and physiological limitations). In real word, however, there is a large number of external constraints (e.g. competitor, predation, etc.) limiting the breadth of the fundamental niche. A subset of the abstract fundamental niche under the presence of all interactions with other species is termed the "realized niche", which is usually narrower than the fundamental one (Hutchinson, 1957).

As formulated by Hutchinson, the niche of a species involves each of the all dimensions (abiotic and biotic) as well as a combination of them, and/or the interactions among different species within communities (Hutchinson, 1957; Kylafis & Loreau, 2011). In practice, however, describing the niche of a species is often a bit difficult due to potentially infinite dimensions. Thus it may be hard to find the significant niche axes of the species without a good understanding of its biology and ecology. Consequently, few intergrative variables may ofen be sufficient to separate species' realized niches among ecologically similar or competing species within communities. These variables are habitat segregation, food resources specialization and/or temporal differentiation (Chesson, 2000; Tokeshi & Schmid, 2002; Kronfeld-Schor & Dayan 2003; Nosil & Sandoval, 2008).

1.3 Niche differentiation

In heterogeneous environments, niche differentiation (synonymous with niche segregation, niche separation or niche partitioning) has long been considered as a major mechanism allowing the maintenance of biodiversity at different scales (Chesson 2000; Chase & Leibold 2003; Leibold & McPeek, 2006). For stable coexistence in competition models, species must differ their responds to, and/or effects on, the environment, including resources they share and all other factors that potentially influence population growth and fitness (Chase & Leibold, 2003). The limiting similarity in Hutchinson's niche model also predicts that the realized niche of certain species is exclusively compared with the others within communities. There is no complete overlap in the realized niches between species and two species can mere share a single realized niche in a stable environment use (Hutchinson, 1957). However, proportional overlap in realized niche is possible, i.e. species may not differ at trophic resources but can differ at microhabitat (Chesson, 2000; Amarasekare, 2003). Therefore, species with little niche overlap along one or two axes can probably allow a long-term coexistence, but somewhat larger overlap can not.

Coexistence among competing species can be certainly maintained by niche separation, but how niche differentiation arises among closely related species is poorly know now? Interspecific competition is widely viewed to be one of the main forces driving a separation in ecological niches to limit niche overlap (Tokeshi & Schmid, 2002; Kylafis & Loreau, 2011). Niche differentiation can arise from current competition (The Ghost of Competition Present), past extinctions (The Ghost of Competition Past) and evolving differences (Morris, 1999; Tilman, 2004; Nosil & Sandoval, 2008; Miller *et al*., 2009). These competition processes eventually result in niche separation and resources partitioning between competing species in a local community, and thus allowing long-term coexistence (Chesson, 2000; Chase & Leibold, 2003).

Since an ecological niche is *n*-dimensional hyper-volume, the niche differentiation involves a very large number of possible abiotic and/or biotic factors, of which the three basic dimensions are spatial (i.e. species may differ in terms of *where* they experience and respond to limiting factors), trophic (i.e. species can differ in terms of *what* they exploit from the same environment), temporal (i.e. species can differ in terms of *when* they respond to the same limiting factors or exploit the same resources) and/or a combination of them (Schoener, 1974; Ross, 1986; Jones *et al.*, 2001; Amarasekare, 2003; Kronfeld-Schor & Dayan, 2003; Leibold & McPeek, 2006). Generally, habitat dimension is more important than the trophic dimension and then the temporal dimension (Schoener, 1974). However, Ross (1986) reviewed the resource partitioning patterns among seven global habitats (tropical reefs, temperate reefs, coastal marine, the Antarctic, mesopelagic/slope environments and freshwater streams and lakes) and concluded that, unlike in terrestrial systems, trophic separation is more important than habitat separation in fish assemblages. Among 37 studies that concurrently examined the niche differentiation along spatial, tropic and temporal axes, he found that about 57% niche showed the greatest separation along food, 32% along habitat, and 11% along time (Ross, 1986).

Nature is strikingly patchy in space and there is always a significant level of spatial heterogeneity in species distribution and abundance (Chesson, 2000; Jones *et al.*, 2001). Organisms that live in different habitats or microhabitats have no direct or indirect interactions with each other and they can coexist stably for a long term even if they may share the same niche (Schoener, 1974; Ross, 1986; Chesson, 2000). Environmental heterogeneity in space is, therefore, a sufficient condition for discreteness of ecological niches. Niche differentiation along space (i.e. habitat segregation) has often been implicated as one of primary mechanism for coexistence among sympatric species with niche overlap (Chesson, 2000; Nicastro *et al.*, 2010). Several studies on taxonomically related or ecologically similar aquatic species have contributed substantially to our understanding the role of spatial segregation in resources partitioning and communities structuring (Cooper *et al.*, 2008; Hernaman &

Probert, 2008; Nicastro *et al*., 2010). Moreover, as diet compositions of a consumer have substantial implications not only for its biology but also for its impacts on sympatric species and community structure, niche differentiation along food resources (i.e. trophic niche segregation) plays an important role in promoting ecological differentiation and maintaining species diversity (Chesson, 2000; Kylafis & Loreau, 2011). Although theory suggests that niche differentiation along temporal (i.e. temporal partitioning) also be an important strategy mediating ecological interactions and facilitating coexistence among co-occurring competitors, its role in structuring communities has often been overlooked (Kronfeld-Schor *et al*., 2001; Kronfeld-Schor & Dayan, 2003). However, a growing number of studies have accumulated and provided empirical evidences of temporal partitioning between competing species such as ants (Albrecht & Gotelli, 2001), bats (Adams & Thibault, 2006), mice (Jones *et al*., 2001; Gutman & Dayan, 2005) and birds (Veen *et al*., 2010). In fish assemblages, however, the role of the niche separation along temporal axis has received less attention (Alanärä *et al*., 2001).

1.4 Ecological invasion of goby species

Invasive species are one of the greatest threats to global biodiversity (Butchart *et al*., 2010; Vörösmarty *et al*., 2010) and the impacts of invasive specie are especially widespread in freshwater ecosystems that are particularly vulnerable to biological invasions (Vörösmarty *et al*., 2010; Cucherousset & Olden, 2011). Fishes are among the most widely introduced group of aquatic animals and the 10 most frequently introduced aquatic species are all freshwater fishes (García-Berthou *et al*., 2005; Gozlan, 2008). The overall number of introduced fishes worldwide from known sources reaches 624 and the rate of non-native freshwater fishes introduced has doubled in the past 30 years (Gozlan, 2008; Gozlan *et al*., 2010). Since many invasive fishes have significant ecological, evolutionary and/or economic impacts on recipient ecosystems, they are increasingly recognized as one of the leading threat to recipient ecosystems, including reduction in native fish abundances, genetic impacts (i.e. hybridization and introgression), alteration of recipient ecosystems, and dissemination of pathogens (Cucherousset & Olden, 2011; Gozlan *et al*., 2010).

Gobiidae is one of the largest families of fishes with more than 2000 species. Many goby species have been widely introduced and become established out of their native ranges including in North American (Dillon & Stepien, 2001; Copp *et al*., 2005), in Europe (Copp *et al*., 2005) and in Asia (Du *et al*., 2001, Yuan *et al*., 2010). In the Great Lakes, for instance, several goby species native to the Ponto-Caspian area have spread to all five lakes, notably the round goby *Apollonia melanostoma* (Pallas, 1814), the tubenose goby *Proterorhinus semilunaris* (Heckel, 1837) and the racer goby *Neogobius gymnotrachelus* (Kessler, 1857) (Dillon & Stepien, 2001). In European, some goby species have also invaded new areas and continue to establish new populations, including the round goby, the bighead goby *Neogobius Kessleri* (Günther, 1861), the monkey goby *Neogobius fluviatilis* (Pallas, 1814) and the racer goby (Copp *et al*., 2005). In Asia, freshwater gobies of the genus *Rhinogobius* (Gill 1859) are frequent benthic fish fauna in most East Asia countries, including China, Korea, Japan and some other regions of south-eastern Asia, such as Philippines, Vietnam and Thailand (Chen & Shao, 1996; Sone *et al*., 2001; Wu & Zhong, 2008). There are at least 17 nominal species of *Rhinogobius* in China (Wu & Zhong, 2008). The introduced gobies of genus *Rhinogobius* have established in most lakes of Yunnan-Guizhou Plateau (Xie *et al*., 2001, Yuan *et al*., 2010). The rapid proliferation of these species has raised serious concerns over their long-term negative impacts on native species and ecosystems (Xie *et al*., 2001; Cooper *et al*., 2008; Yuan *et al*., 2010; Kornis *et al*., 2012). The most notorious species is the round goby, one of the most wide-ranging invasive fish on earth, which has widely introduced into Laurentian Great Lakes watershed, the Baltic Sea and several major European rivers and has showed many nagetive effects on ecosystems (Kornis *et al*., 2012). The invasive round goby affects many native species through resource competition, spawning interference and displacement of native species to sub-optimal habitat (Kornis *et al*., 2012). In the Great Lakes, it has resulted in several ecological effects such as alteration of nutrient and contaminant pathways, and increasing outbreaks of avian botulism (Ng *et al.*, 2008; Kornis *et al.*, 2012). However, few studies have paid attention to specific management strategies for the invasive goby species (Gozlan *et al.*, 2010; Kornis *et al.*, 2012).

Because of the wide and significant ecological and economic impacts of no-native fishes on recipient ecosystems (many of those impacts are detrimental), an increasing attention has been devoted to the management of invasive fishes (e.g. preventing their further invasion, mitigating their negative ecological effects, etc.) during the last decade (Sutherland *et al.*, 2009; Gozlan *et al.*, 2010). Several measures (e.g. physical removal, chemical eradication and bio-manipulation) have been developed and implemented to control, contain or eradicate a wide range of invasive species (Taylor & Hastings, 2004; Britton *et al.*, 2011). The effectiveness of these measures is, however, highly dependent upon the life, biological and ecological characteristics of the targeted invasive species (Ludgate & Closs, 2003; Taylor & Hastings, 2004; Britton *et al.*, 2011; Yeates *et al.*, 2012). For instance, the egg of Salmonids are around 100 times less sensitive to rotenone than juvenile and adult (Marking & Bills, 1976), and therefore eradications using rotenone should account for different vulnerabilities among life stages (Ling, 2002; Britton *et al.*, 2011). Therefore, a full understanding of the life characteristics of invasive species is crucial to develop economically and/or ecologically effective management strategies (Ludgate & Closs, 2003; Taylor & Hastings, 2004; Yeates *et al.*, 2012).

Watersheds of the Yunnan Province is one of the highest biodiversity hotspots in China and there are 432 documented freshwater fish species, accounting for 42.2% of the total freshwater fish species of China (Xie *et al.*, 2001). However, many invasive fish species have been introduced since the 1950-60s and a large number of native fish species (about one third of 432 native fish species) have become endangered or extinct (Xie *et al.*, 2001; Yuan *et al.*, 2010). For instance, seventeen native fish species inhabited in Lake Erhai in the 1950s (Du & Li, 2001). Currently, there are only seven native species coexisting with twenty-two introduced species. The most notorious introduced fish species in lakes of the Yunnan Province are small-bodied goby of the genus *Rhinogobius* (Gill 1859) and *Neosalanx taihuensi*s (Chen, 1956), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873), *Hemiculter leucisculus* (Basilewsky, 1855) and *Abbottina rivularis* (Basilewsky, 1855) (Yuan *et al*., 2010). Although significant attention has been paid locally to these species, the control actions have minimal success or require indefinite investments and the outcomes are often discouraging (Du & Li, 2001; Yuan *et al*., 2010). An important fact overlooked here was that most of these management strategies were implemented without an appropriate knowledge of life, biological and ecological characteristics.

1.5 Specific objectives

Rhinogobius giurinus (Rutter, 1897) and *Rhinogobius cliffordpopei* (Nichols, 1925) are frequent species (native species) in lakes along the middle and lower reaches of the Yangtze River (Xie *et al*., 2000a; Xie *et al.*, 2005; Zhang, 2005; Li *et al*., 2010). They were inadvertently introduced into most lakes of the Yunnan-Guizhou Plateau in the 1950-60s and introduced simultaneously to Lake Erhai in 1961 (Du & Li, 2001; Xie *et al*., 2001; Yuan *et al*., 2010). Since then they have become the most dominant benthic fish species with the annual yield accounting for 48 % of total fish yields (kg) of the lake in 2010 (unpublished data). In native lakes along the middle and lower reaches of the Yangtze River, they are ecologically similar species with comparable life histories, such as small-sized (maximal total body length < 80 mm), reproducing in spring and early summer, zooplanktivorous, etc. (Xie *et al*., 2000a; Xie *et al.*, 2005; Zhang, 2005). The present study focuses on the mechanisms that allow a long-term coexistence of two congeneric and competing goby species within this lake. Consequently, habitat selections, life characteristics, diet compositions and feeding behaviors of the two highly abundant and ecologically similar invasive goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) were compared to test whether there was a niche separation (involving spatial, trophic and temporal axes) allowing their stable coexistence in the lake.

Specifically, in the study of habitat selections (i.e. niche separation along spatial axis), the objectives were to determine whether spatial segregation occurs between the two species by examining how their abundances differed across the habitats and how their abundances were associated with environmental characteristics. In the study of food resources exploitation (i.e. niche separation along trophic axis), our aims were to determine whether the trophic niche (food resources) of the two species differed in each habitat and how their trophic niche changed with habitats as well as life stages during ontogeny. In the study of life characteristics (age, growth, reproduction biology and population dynamic) and feeding behaviors, our objectives were to investigate temporal partitioning (i.e. niche separation along temporal axis) in terms of spawning cycles, appearance of similar-sized cohorts, population dynamics, diel activities and feeding rhythms.

Moreover, the two species mainly prey on large zooplankton (Cladocera and Copepoda) and aquatic insects (Chironomid larvae), and they often prey on fish larvae and eggs, including those of native species (Xie *et al*., 2000a; Du *et al*., 2001; Zhang, 2005). They are widely considered to compete with native species (e.g. *Cyprinus longipectoralis* (Chen & Huang, 1977), *Cyprinus barbatus* (Chen & Huang, 1977), *Barbodes daliensis* (Wu & Lin, 1977)) for food resources (Du *et al*., 2001) and reduce the native species especially when their populations are highly abundant in the lake. The two species are therefore thought to be one of the major causes of the decline and/or extinction of most native fishes in the lake (Du *et al*., 2001). Currently, the principal management strategy to control the two species in the lake is the removal by the local fishery, but the outcomes are discouraged as the populations of those two gobies have become more dominant in the latest years. Thus, based on their habitat selections and life characteristics (i.e. growth pattern, reproduction biology and population dynamic), this study aimed at providing specific strategies to improve the cost-efficiency of the current management programs for the two pest species.

2 Materials and Methods

2.1 Study site and habitat characteristics

Lake Erhai (105°5-17′ E, 23°35-58′ N) is a freshwater plateau lake in Yunnan-Guizhou Plateau of China (Fig. 1). Its surface area is about 250 km^2 and catchment area is about 2,600 km^2. The maximum water depth is approximately 21 m without thermal or dissolved oxygen stratification. Water temperature usually peaks at around 25-27 °C in July or August and drops to approximately 6 - 8 °C in December or January. Currently, twenty-eight fish species have been found in the lake in recent years and most of them are planktivorous. The dominant species are small-bodied fishes, especially non-native species, including freshwater gobies *Rhinogobius giurinus* and *R. cliffordpopei*, *Neosalanx taihuensis* (Chen, 1956), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873) and *Hemiculter leucisculus* (Basilewsky, 1855). *Channa argus* (Cantor, 1842) is the only recorded piscivorous fish species.

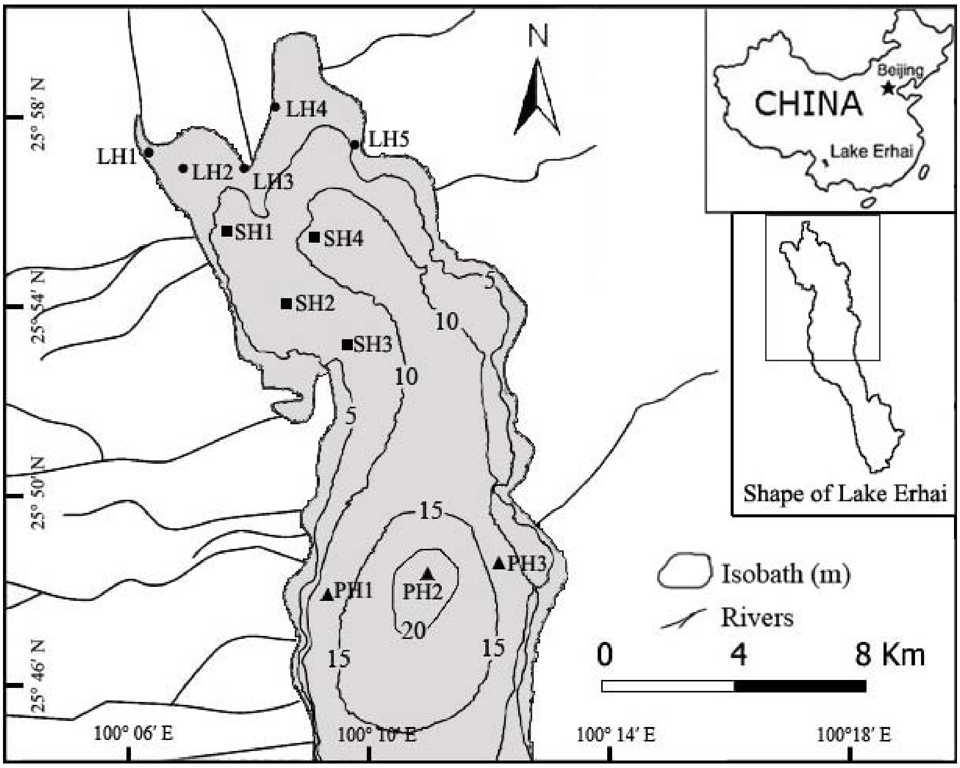

Fig. 1 Sampling sites in the three habitats located in the central and northern section of Lake Erhai (China). Five (LH1-LH5), four (SH1-SH4) and three (PH1-PH3) sites were sampled in littoral (●), sub-littoral (■) and profundal (▲) habitats, respectively.

Habitat characteristics of the lake were investigated prior to fish sampling in 2010 (Table 1). Habitats for benthic fishes in Lake Erhai were classified into three types: 1) littoral habitat (LH, water depth less than 6 m, high abundance of submerged macrophytes, submersed-macrophyte detritus substrata); 2) sub-littoral habitat (SH, water depths range 6 to 12 m, few submerged macrophytes, submersed-macrophyte detritus and silt substrata); and 3) profundal habitat (PH, water depths ranges 12 to 20 m, no submerged macrophytes, silt and coarse sand substrata, Fig. 1 & Table 1). There were Five (LH1-LH5), four (SH1-SH4) and three (PH1-PH3) sites in each habiat (Fig. 1 & Table 1). For each sampling site, water temperature, dissolved oxygen and conductivity were measured *in situ* using a handheld meter (YSI Model Pro20, Ohio, USA). A handheld pH meter was used to measure pH *in situ* (YSI Model EcoSense pH10A, Ohio, USA). Total nitrogen, total phosphorus and Chlorophyll *a* were determined using a standard colorimetric method (APHA *et al*., 1995). Macrozooplanktons were collected by hand-nets (mesh size 64 μm) and counted under a dissecting microscope. Substrata structures were sampled by Peterson dredge (0.0625 m^2) and determined by macroscopic appearances. Biomass (wet weight) of submerged macrophytes was determined using a 30 cm × 50 cm clamp at three random measurements within each sampling site.

Table l Environmental variables measured in the three habitats in Lake Erhai in 2010 (China). Reported values are mean ± standard deviation.

Environmental variables	Littoral habitat (LH)	Sub-littoral habitat (SH)	Profundal habitat (PH)
Water depth (m)	3.11± 1.17 (n=40)	8.13 ± 1.35 (n=32)	15.92 ± 3.14 (n=24)
Water temperature (°C)	16.92 ± 4.74 (n=40)	17.14 ± 4.48 (n=32)	16.91 ± 4.64 (n=24)
Submerged macrophytes (g m^{-2})	3307.35 ± 881.32 (n=40)	102.66 ± 49.49 (n=32)	0 (n=24)
pH	9.33 ± 0.30 (n=40)	9.20 ± 0.33 (n=32)	9.08 ± 0.26 (n=24)
Secchi depth (cm)	214.17 ± 35.91 (n=40)	235.43 ± 22.47 (n=32)	254.66 ± 25.43 (n=24)
Conductivity (μScm^{-1})	237.33 ± 167.50 (n=40)	232.62 ± 11.89 (n=32)	233.87 ± 12.76 (n=24)
Dissolved oxygen (mg L^{-1})	9.23 ± 1.05 (n=40)	7.56 ± 1.08 (n=32)	7.51 ± 0.88 (n=24)
Total nitrogen (mg L^{-1})	0.46 ± 0.12 (n=20)	0.42 ± 0.08 (n=16)	0.43 ± 0.05 (n=12)
Total phosphorus (mg L^{-1})	1.38E-2 ± 0. 81E-2 (n=20)	1.26E-2 ±0.77E-2 (n=16)	1.42E-2 ±0.69E-2 (n=12)
Chlorophyll *a* (mg L^{-1})	8.27 ± 2.88 (n=20)	12.12 ± 3.82 (n=16)	12.44 ± 3.09 (n=12)
Macrozooplankton (ind. L^{-1})	296.43 ± 60.56 (n=20)	198.62 ± 35.48 (n=16)	92.65 ± 27.43 (n=12)
Substrata structures [1]	-1	0	1
CF [2]	32.91 ± 8.16 (n=160)	7.67 ± 2.74 (n=128)	0.16 ± 0.09 (n=96)

[1] Type of submersed macrophyte detritus, mixture of submersed macrophyte detritus and silt, mixture of silt and coarse sand were assigned value of -1, 0 and 1, respectively. [2] Total abundances (ind. net^{-1} day^{-1}) of all fish species except *R. giurinus* and *R. cliffordpopei* caught in fyke nets.

2.2 Fish sampling and data collection

2.2.1 Habitat selections

Five (LH1-LH5), four (SH1-SH4) and three (PH1-PH3) sites were sampled for each habitat in the middle and northern section of the lake, respectively (Fig. 1 & Table 1). Fish sampling was carried out in the first week of February (winter), May (spring), August (summer) and November (autumn) of 2010 using benthic fyke nets. The net comprised a trunk stem with twenty traps, two end traps and two end pockets. Total length of the net was 15 m, including 12 m of trap (0.6 m for each trap), 2 m of end trap (1 m for each trap) and 1 m of end pocket (0.5 m for each one). The framework of each trap was made of iron wire with the width of 0.35 m and the height of 0.62 m. The end trap was round and the diameter gradually decreased from 0.3 m to 0.1 m. The mesh size of all nets was 4 mm. At each sampling site, eight nets were deployed separately with a stone in each end of the nets. After 24 hours, the catches in the end pockets were collected and transferred to -20 °C. In each season, the nets were deployed at 8:00 to 10:00 am and we sampled three sites per day (twenty four nets were used each day). The order of site sample was identical in the four seasons. Hence, fish sampling lasted four days in each season. All fishes collected in each fyke net were identified to the species level, counted and batch-weighed. The procedures were performed following the legislation in China (GB14925-2010).

2.2.2 Diet compositions and ontogenetic diet shift

In PH and SH, *R. cliffordpopei* and *R. giurinus* used for trophic analyses were collected monthly from May to August in 2010 using benthic fyke nets (Fig. 1). The diet compositions were analyzed using gut content analyses (GCA) and stable isotope analyses (SIA). The individuals sampled for GCA were preserved in 8 % formalin for two weeks and then transferred to 75% ethanol for storage. The individuals sampled for SIA were stored at -20°C.

For gut content analyses (GCA), total length (TL, nearest mm) and body weight (BW, nearest 0.01 g) of each specimen were measured. Since there was no clear stomach for the two species, the fore-gut, which was defined as the section of intestine from the oesophagus to the posterior end of the first loop, was sampled for diet analyses (Xie *et al.*, 2000a; Xie *et al.*, 2005). Fore-gut contents of non-empty guts were removed and preserved in 2 ml plastic tube with 75 % ethanol. Food items were identified to the lowest possible taxonomic level under a dissecting microscope. The weight of Cladocera and Ostracoda were calculated as 0.023 mg per individual. The weight of Copepoda was calculated as 0.014 mg per individual. The weight of Copepoda larva was calculated as 0.003 mg per individual (Zhang, 2005). The weight of fish eggs, plant materials and unidentified items were roughly equal to their volumes (specific gravity was assumed to be 1). After removing surface ethanol by blotting them on tissue paper, shrimp larvae, aquatic insects, Gastropoda and fish larvae was weighted to the nearest 0.1 mg (Hyslop, 1980). For each prey category, frequency of occurrence (%O), percentage of number (%N) and percentage of weight (%W) were calculated as: %O*i* = 100 O*i* / $\sum_{1}^{n} Oi$, where O*i* is the number of the guts that contain food category *i*), %N*i* = 100 N*i* / $\sum_{1}^{n} Ni$, where N*i* is the number of food category *i* and %W*i* = 100 W*i* / $\sum_{1}^{n} Wi$, where W*i* is the weight of food category *i*) (Hyslop, 1980). Food compositions of the two goby species were finally estimated using index of relative importance (%IRI) that was calculated as formula: %IRI = 100 IRI*i* / $\sum_{1}^{n} IRIi$, where IRI*i* = (%N*i* + %W*i*) / %O*i* (Assis, 1996). Based on their life characteristics, specimens of the two species used for GCA were grouped into three life stages, i.e. juveniles (gonados are at stage I, gonados are indistinguishable for sexes by naked eye), sub-adults (gonados are at stage II to III) and adults (gonados are at stage IV to V). In LH, LT (mean ± SD, mm) of juveniles, sub-adults and adults for *R. cliffordpopei* were 14.7 ± 3.1 (n=27), 27.4 ± 3.9 (n = 35) and 41.3 ± 6.2 (n = 41), respectively, and for *R. giurinus* were 15.4 ± 3.4 (n=34), 36.1 ± 2.9 (n=42) and 50.4 ± 6.4 (n=49), respectively. In PH, LT of juveniles, sub-adults and adults for *R. cliffordpopei* were 15.1 ± 2.5 (n=29), 29.9 ± 3.1 (n = 25) and 43.1 ± 5.6 (n = 41) respectively, and for *R. giurinus* were 14.9 ± 3.2 (n=37), 37.3 ± 5.7 (n=33) and 53.4 ± 4.7 (n=30), respectively. In addition, because the preservation can affect the length/weight ratio, all subsequent analyses were performed based on preservation-corrected TL and BW. Specifically, TL and BW were measured individually before and after preservation for 100 specimen and the relationships between fresh and preserved TL and BW were established (TL_{Fresh} = 1.0066 $TL_{Preserved}$ + 0.4174, R^2 = 0.9559 and BW_{Fresh} = 0.8771 $BW_{Preserved}$ + 0.0112, R^2 = 0.9668, n = 100).

To determine the stable isotope values of potential food resources in each habitat, macrozooplankton, Tubificidae, aquatic insects and shrimp larvae were sampled in May 2010. Macrozooplankton were Cladocera (dominated by Daphniidae, Chydoridae and Bosminidae) and Copepoda (dominated by Cyclopoida). Tubificidae were dominated by *Limnodrilus* and aquatic insects were dominated by Chironomidae. Shrimp larvae were sampled using benthic fyke nets and dominated by Atyidae (body mass was 75.5 ± 21.9 mg, mean ± SD). Macrozooplankton, Tubificidae and aquatic insects were kept alive in distilled water for 24 hours to clean gut contents, killed by freezing, and then subjected to SIA. Shrimp larvae were kept at -20°C.

In stable isotope analyses (SIA), dorsal muscles of each individual of the two gobies and shrimp larvae were used for SIA. Dorsal muscles of the individuals with the same LT were pooled. These dorsal muscles and potential food resources (i.e. macrozooplankton, Tubificidae, aquatic insects and shrimp larvae) were oven dried at 60 °C for 48 hours and ground into homogeneous powders using a mortar and pestle. SIA were performed using an Elemental Analyzer (Flash EA1112 HT) along with an Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific，Inc., USA). Isotopic ratios were presented as relative to international standards (peedee belemnite carbonate (C) and atmospheric nitrogen (N)) in delta (δ) notation. Standard deviation (SD) of replicated samples was less than 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$. In LH, LT (mean ± SD, mm) of juveniles, sub-adults and adults for *R. cliffordpopei* were 13.1 ± 4.0 (n=10), 32.9 ± 3.6 (n=12) and 41.8 ± 5.7 (n=13), respectively, and for *R. giurinus* were 14.4 ± 3.3 (n=14), 35.5 ± 4.5 (n=24) and 52.1 ± 8.4 (n=16), respectively. In PH, LT of juveniles, sub-adults and adults for *R. cliffordpopei* were 13.6 ± 4.8 (n=12), 30.2 ± 4.8 (n = 13) and 44.1 ± 6.4 (n=10) respectively, and for *R. giurinus* were 14.2 ± 3.6 (n=10), 37.5 ± 6.4 (n=20), 54.8 ± 8.5 (n=21), respectively.

2.2.3 Age, growth, reproduction and population dynamics

The gobies were caught monthly using fyke nets from October 2009 to October 2011 in LH and PH (Fig. 1). In each month, we pooled all individuals of *R. cliffordpopei* from the five sampling sites in LH and *R. giurinus* from the three sampling sites in PH. Random sub-samples (ranging 400-800 individuals for each species) from the pool were taken and kept in a refrigerator for subsequent analyses.

For each month, the scales of thirty individuals were sampled randomly for age determination. The sexes of about 200-300 individuals from the sub-samples were determined based on the macroscopic appearances of gonads following Yan & Chen (2007) (Table 2). The scales were immersed in 10 % NaOH for three hours, cleaned with running water and then read under an optical microscope using the criteria of Steinmetz & Müller (1991). Based on macroscopic appearances, gonads of both sexes were classified into five reproductive stages (stage I to V, Table 2). Developmental stages of ovaries and testes were assessed by visual inspection primarily based on its appearances (Table 2). Gonads (≥ stage II, Table 2) were carefully removed and weighed to the nearest 0.l mg (gonad mass, MG).

Table 2 Macroscopic characteristics (modified from Yan & Chen (2007)) used to classify gonad maturity stages of *R. cliffordpopei* and *R. giurinus* in Lake Erhai (China)

Gonad maturity stages	Female	Male
I (virgin stage)	Sexes are indistinguishable by naked eye; gonads are thin, threadlike and transparent	Sexes are indistinguishable by naked eye; gonads are thin, threadlike and transparent
II (immature stage)	Ovaries are small, translucent or pale-yellow, more rod-shaped than stage-I; oocytes are indiscernible	Testes are lender, white or gray and larger than stage-I s
III (maturing stage)	Ovaries are swelling, orange and occupy 1/5 to 1/3 of body cavity; vitellogenic oocytes are tightly packed in ovaries and can be visible from epithelium	Testes are firm, flat-shaped and ivory white
IV (spawning stwage)	Ovaries occupy 1/3 to 2/3 of body cavity; oocytes attain their maximum volume and can be released from genital pore with light abdominal pressure	Testes are ivory white and milt can be released genital pore with light pressure on the abdomen
V (spent stage)	Ovaries are flaccid and sometimes red with visible capillaries; there are often a few of residual oocytes in ovaries	Testes are flaccid and deceased volume clearly; milt are found in some individuals

2.2.4 Diel activity and feeding rhythm

R. cliffordpopei and *R. giurinus* were sampled using benthic fyke nets in spring (April), summer (July), autumn (October) and winter (January) in 2011 in SH (Fig. 1). In each season, the gobies were collected once every two hours for 24 hours of a day. Five nets were used for each time. The nets were deployed at 7:00, retrieved at 8:45 and deployed again at 9:00 for a next round. From the second time, the sample site was moved randomly and was proximately two hundred meters away from the previous one. At each time, all gobies in the end pockets of the nets were collected and counted. The specimens were preserved as described above. The fore-guts were identified using a dissecting microscope. After removing surface ethanol by blotting them on tissue paper, the non-empty guts were weighed to the nearest 0·01 mg, then food contents were removed and the emptied guts were reweighed.

2.3 Data calculation and statistical analyses

2.3.1 Habitat selections

Goby abundances in the three habitats were estimated using catch per unit effort (CPUE), defined as the number of individuals caught in one net per day (ind. net^{-1} day^{-1}). Differences in goby abundances (i.e., CPUE) between habitats and seasons were tested using repeated-measures ANOVA (RM-ANOVA) with habitats as a fixed factor and seasons as a random factor. Sphericity assumption was tested using Mauchly’s test and the degrees of freedom were adjusted by Greenhouse–Geisser Epsilon when data violated the assumption of sphericity. Because the interactions between habitats and seasons were significant, one-way ANOVAs with Tukey’s multiple comparisons were subsequently used to test the differences in goby abundances between habitats in each season separately. Data of goby abundances were log-transformed to achieve normality and homoscedasticity. Correlation analyses with Pearson correlation coefficients were used to explore how goby abundances were associated with environmental variables. Correlation analyses were conducted separately for the two goby species. Environmental variables and goby abundances were averaged for each season.

2.3.2 Diet compositions and ontogenetic diet shifts

Chi-square test (χ^2 test) was used to test the differences in food compositions (%IRI) between life stages (juveniles, sub-adults and adults) within species, and between species (*R. cliffordpopei* and *R. giurinus*) and habitats (LH and PH) for the same life stage.

The stable isotopic values of food resources showed no significant differences between LH and PH (Mann-Whitney tests, $P > 0.05$). After examination of normality and variances homogeneity of data using Kolmogorov- Smirnov and Levene's tests, three-way analyses of variance (ANOVA) were used to test the differences of stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$ were the inverse transformed and tested separately) between life stages, species and habitats. If species showed significant effects on stable isotopic values, Mann-Whitney tests were used to test for differences between species within habitat for the same life stage.

The contributions of potential food resources to the diet of the two species were estimated using stable isotope mixing models (Stable Isotope Analysis in R; Parnell *et al*., 2008). Since there are no available trophic fractionation factor (TEF) values for the two goby species, we used an averaged TEF with a large SD as suggested by Inger *et al*. (2010). Here, the input TEF was 1.0 ± 1.0 ‰ (mean ± SD) for $\delta^{13}C$ and 3.3 ± 1.0 ‰ (mean ± SD) for $\delta^{15}N$ (Inger et al., 2010). Mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD, n=3) of potential food resources in each habitat were used in the models. As life stages and habitats significantly affected the values of $\delta^{13}C$ and $\delta^{15}N$, the models were run separately for different life stages in each habitat. Finally, the predicted percentage of contribution (mean ± SD) of each food source to the diet of the two species at the three life stages was averaged.

2.3.3 Age, growth, reproduction and population dynamics

Age was determined by reading scales. Monthly body length-frequency was used to analyze cohort structures. In the same month, Kolmogorov-Smirnov (K-S) test was used to test differences in body length-frequency between males and females within species, and between species for the entire populations (including males, females and those unidentifiable for sexes).

Growth patterns were first estimated using monthly changes in mean LT and body condition. Body condition was the *b* estimated by a linear regression between

LT and MT: $\log_{10}$ MT = a + b $\log_{10}$ LT. The mean LT and body condition were compared between males and females within species, and between species for the entire populations using Mann-Whitney test and analyses of covariance (ANCOVAs) with LT as a covariate, respectively. Second, growth patterns were described by von Bertalanffy growth function (von Bertalanffy, 1938): $L_t = L_\infty [- e^{-K (t - t_0)}]$, where L_t is the total body length at the time t, L_∞ is the asymptotic length, K is the von Bertalanffy growth coefficient, t_0 is the theoretical age at $L_T = 0$. VBGF was only estimated for the entire populations because the sexes of most individuals were unidentifiable by macroscopic appearances of gonads in five months of a year. The function parameters were estimated using Electronic Length Frequency Analysis (ELEFAN) in the FiSAT II package (FAO – ICLARM Fish Stock Assessment Tools, Version 1.2.2, Gayanilo *et al.*, 2005). t_0 is was calculated as $\log_{10} (-t_0) = -0.392 - 0.275 \log_{10} L_\infty - 1.038 \log_{10} K$ (Pauly, 1979).

Reproduction cycles were estimated using gonado-somatic indices (I_G) of females, which were calculated for each mature specimen using the formula $I_G = 100\ M_G / M_T$. The onset and duration of reproduction seasons were quantified based on the occurrence of individuals with stage-IV ovaries. The intensity of reproduction activities was determined by comparing I_G and the proportions of different ovary developmental stages between sampling dates. Logistic regression models were then used to quantify the proportion of mature males and females (stage IV and V,) at any L_T using binomial maturity data (immature 0, mature 1). L_T at which 50 % of individuals are sexually mature was defined as the mean size of maturity (L_{m50}). A log-likelihood ratio tests were used to test the differences of L_{m50} between sexes within each species. Mann-Whitney tests were used to test for potential differences in LT between males and females within each species.

Population dynamics of the two species were described using mortality and catch per unit effort (CPUE) of the fyke nets. Specifically, mortality calculations were conducted using the procedures in FiSAT II package (Gayanilo *et al.*, 2005). Total mortality (Z, i.e. the sum of natural and fishing mortality) was estimated using length-converted catch curves (Pauly, 1983). This method pools a long series of samples that represent a steady-state population and generate a single frequency distribution accounting for their relative importance. Z is then calculated on the descending part of this single global distribution (Pauly, 1983). Natural mortality (M) is correlated with asymptotic length (L_∞), the von Bertalanffy growth coefficient (K), and mean environmental temperature (T) by the Pauly's M equation (Pauly, 1987): $\ln(M) = -0.015 - 0.279 \ln(L_\infty) + 0.654 \ln(K) + 0.463 \ln(T)$. The mean annual temperature used for M estimates was 18.7 °C. It was averaged from the monthly temperatures of Erhai Lake from October 2009 to October 2011. Fishing mortality (F) was then obtained by subtracting M from Z. The exploitation ratio (E) was calculated as $E = F/Z$.

Finally, catch per unit effort (CPUE, ind. net^{-1} day^{-1}) of the fyke nets were calculated monthly and defined as the number of goby species caught in one net per day. Differences of CPUE (log-transformed) between species and months were tested using repeated-measures ANOVA (RM-ANOVA) with species as a fixed factor and months as a random factor. Sphericity assumption was tested using Mauchly's test and the degrees of freedom were adjusted by Greenhouse–Geisser Epsilon when data violated the assumption of sphericity. In the same month, Mann-Whitney was subsequently used to test the differences in CPUE between species if species have significant effects on CPUE. The same approaches were used to test the differences in CPUE between sexes (fixed factors) within species and months (random factors).

2.3.4 Activity level and feeding rhythm

The diel and seasonal activity were determined using the percentage of catch per unit effort (PCPUE, %) of benthic fyke nets, which was calculated as: 100 × number of individuals caught in a given two hours per net / total number of individuals caught in 24 hours of day per net. The differences in PCUPE (log (x+1) transformed) among seasons and species were tested using Mixed-Effects Models with seasons and species as fixed factors and time of the day as a random factor. Because seasons had significant effects on CPUE, one-way ANOVAs were subsequently used to test the differences in PCPUE between seasons within species. Multiple comparisons among means were conducted using Tukey's HSD *post-hoc* test when significant difference were identified by one-way ANOVAs.

The diel and seasonal feeding rhythm were determined using the percentage of empty gut (PEG) and weight of gut content (WGC). PEG was calculated as: FEG (%) = 100 number of empty guts/ number of total guts. WGC was calculated as: WGC (%) = 100 × food weight of non-empty gut / eviscerated weight of the body. Mann-Whitney test was used to test the differences in PEG among seasons within species, and between the two species within the same season. The differences in WGC (log (x+1) transformed) among seasons and species were tested using Mixed-Effects Models with seasons and species as fixed factors and time of the day as a random factor. The subsequent analyses were the same as used for PCPUE.

Normality and homogeneity of data were determined using one-sample Kolmogorov–Smirnov test and Levene's test. All statistical analyses were performed using R version 2.14.2 (R Development Core Team 2012).

3 Results

3.1 Niche separation along spatial axis

3.1.1 Goby abundances across habitats and seasons

A total of 67,819 individuals of *R. giurinus* and 36,043 individuals of *R. cliffordpopei* were sampled. The abundances of the two goby species were significantly influenced by habitat, season, and the interaction between habitat and season (RM-ANOVA, Table 3 & Fig. 2).

Table 3 Repeated-measures ANOVAs (RM-ANOVA) used to test differences in abundances of *R. giurinus* and *R. cliffordpopei* between habitats (fixed factor) and seasons (random factor) in Lake Erhai (China).

Species	Sources	SS	d. f.	MS	*F*	*P*
R. giurinus	Habitats	111.112	2	55.556	288.436	< 0.001
	Error	17.913	93	0.193		
	Seasons	16.398	1.847	8.877	46.508	< 0.001
	Error	32.791	171.800	0.191		
	Habitats × Seasons	14.093	3.695	3.814	19.984	< 0.001
R. cliffordpopei	Habitats	119.528	2	59.764	221.213	< 0.001
	Error	25.125	93	0.270		
	Seasons	43.810	2.293	19.110	122.403	< 0.001
	Error	33.286	213.209	0.156		
	Habitats × Seasons	26.149	4.585	5.703	36.529	< 0.001

Specifically, the abundance of *R. giurinus* significantly differed between habitats across the four seasons (one-way ANOVAs, Table 4). The abundance of *R. giurinus* was significantly higher in PH than in LH and SH for all seasons (Tukey HDS, $P < 0.001$) and in SH than in LH (Tukey HDS, $P < 0.001$) except in winter (Tukey HDS, $P = 0.079$) (Fig 2). The abundance of *R. cliffordpopei* also significantly differed between habitats across the four seasons (one-way ANOVAs, Table 4). In spring, autumn and winter, the abundance of *R. cliffordpopei* was significantly higher in PH than in SH and in LH (Tukey HDS, $P < 0.001$), whereas there were no significant differences between LH and SH (Tukey HDS, $P = 0.132$, *P*

= 0.095 and P = 0.422, respectively). In summer, the abundance *R. cliffordpopei* was significantly different among the three habitats (Tukey HDS, LH vs SH, P = 0.016, LH vs PH, P < 0.001, SH vs PH, P < 0.001) (Fig. 2).

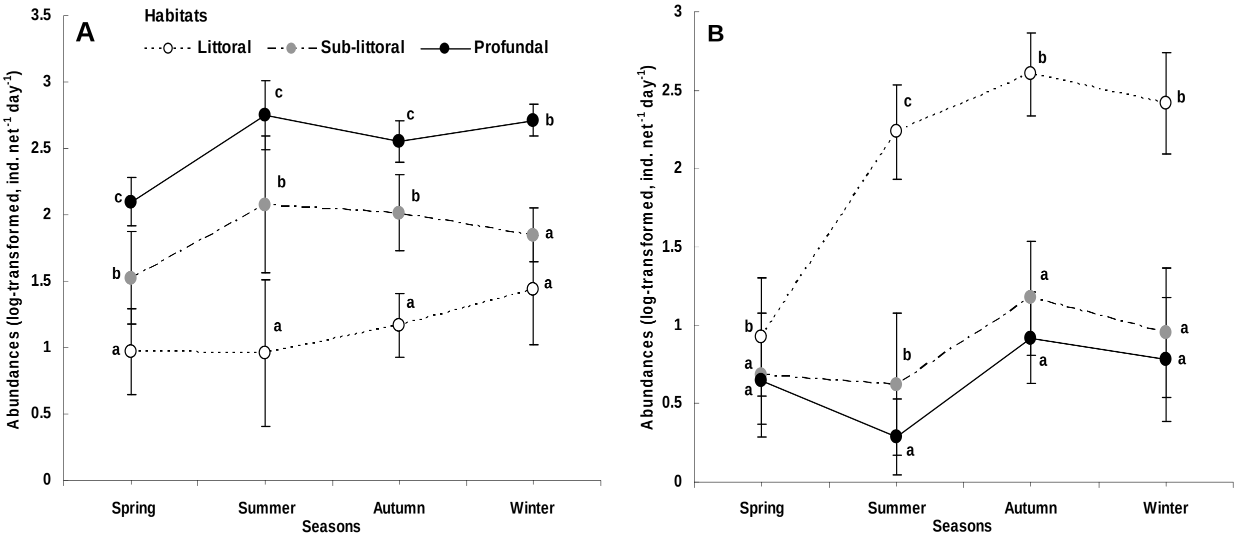

Fig. 2 Mean abundances (log-transformed) of (A) *R. giurinus* and (B) *R. cliffordpopei* across habitats and seasons (sampling sizes were 40, 32 and 24 for littoral, sub-littoral and profundal habitat in each season, respectively) in Lake Erhai (China). The abundances in the same season with different letters were significantly different. Error-bars represent the standard deviations.

Table 4 One-way ANOVAs used to test the differences in the abundances of *R. giurinus* and *R. cliffordpopei* between habitats for each season in Lake Erhai (China).

		R. giurinus					*R. cliffordpopei*				
Seasons	Sources	SS	d. f.	MS	*F*	*P*	SS	d. f.	MS	*F*	*P*
Spring	Between Habitats	51.387	2	25.694	82.014	<0.001	61.219	2	30.609	139.383	<0.001
	Within Habitats	29.135	93	0.313			20.423	93	0.220		
	Total	80.522	95				81.642	95			
Summer	Between Habitats	27.900	2	13.950	239.734	<0.001	41.445	2	20.722	179.449	<0.001
	Within Habitats	5.412	93	0.058			10.739	93	0.115		
	Total	33.312	95				52.184	95			
Autumn	Between Habitats	33.311	2	16.655	216.930	<0.001	41.582	2	20.791	131.972	<0.001
	Within Habitats	7.140	93	0.077			14.651	93	0.158		
	Total	40.451	95				56.233	95			
Winter	Between Habitats	12.607	2	6.303	65.013	<0.001	1.432	2	0.716	5.286	0.007
	Within Habitats	9.017	93	0.097			12.598	93	0.135		
	Total	21.624	95				14.030	95			

3.1.2 Goby abundances in relation to environmental variables

A strong association between environmental variables and goby abundances was observed for the two species. Specifically, Pearson correlation coefficients (r) demonstrated that the abundances of *R. giurinus* were positively associated with water depth ($r = 0.667$, $P < 0.001$), silt and coarse sand substrata ($r = 0.640$, $P < 0.001$). In contrast, the abundances of *R. cliffordpopei* were positively correlated with submerged macrophytes ($r = 0.662$, $P < 0.001$), abundances of other fish ($r = 0.606$, $P < 0.001$), densities of macrozooplankton ($r = 0.363$, $P < 0.001$), concentrations of dissolved oxygen ($r = 0.323$, $P < 0.001$) and submersed macrophyte detritus substrata ($r = -0.608$, $P < 0.001$, Table 5).

Table 5 Correlation analyses (Pearson correlation coefficients (r) and P values) between abundances of *R. giurinus*, *R. cliffordpopei* and environmental variables in Lake Erhai (China).

	R. giurinus		*R. cliffordpopei*	
Environmental variables	r	P	r	P
Water depth (m)	0.677	< 0.001	-0.572	< 0.001
Water temperature (°C)	0.169	0.001	0.174	0.001
Submerged macrophytes (g m^{-2})	-0.448	< 0.001	0.662	< 0.001
pH	-0.053	0.300	0.093	0.068
Secchi depth (cm)	0.209	< 0.001	-0.224	< 0.001
Conductivity (µScm^{-1})	-0.015	0.743	0.051	0.318
Dissolved oxygen (mg L^{-1})	-0.226	< 0.001	0.323	< 0.001
Total nitrogen (mg L^{-1})	-0.089	0.064	0.083	0.104
Total phosphorus (mg L^{-1})	0.013	0.794	0.097	0.084
Chlorophyll *a* (mg L^{-1})	0.188	< 0.001	-0.010	0.894
Macrozooplankton (ind L^{-1})	-0.524	< 0.001	0.363	0.011
CF	-0.452	< 0.001	0.606	< 0.001
Substrata structures	0.640	< 0.001	-0.608	< 0.001

3.2 Niche separation along food axis

3.2.1 Ontogenetic trophic niche shift

The two species showed a strong trophic niche shift during ontogenetic development. The gut content analyses (GCA) indicated that juveniles of the two species showed significantly different diet compositions (%IRI) with sub-adults and adults (χ^2 test, P

< 0.05), whereas diet compositions were similar between sub-adults and adults (χ^2 test, *P* > 0.05, Fig. 3 & Table 6). Specifically, %IRI of macrozooplankton was 50.2 - 71.0 % for juveniles but decreased to 20.4 - 35.5 % for sub-adults and 12.4 - 27.6 % for adults (Fig. 3). %IRI of aquatic insects were less than 9.3 % for juveniles and increased to 20.4 - 55.7 % for sub-adults and 30.6 - 61.3 % for adults. %IRI of shrimp larvae ranged 8 - 19% and varied slightly with life stages.

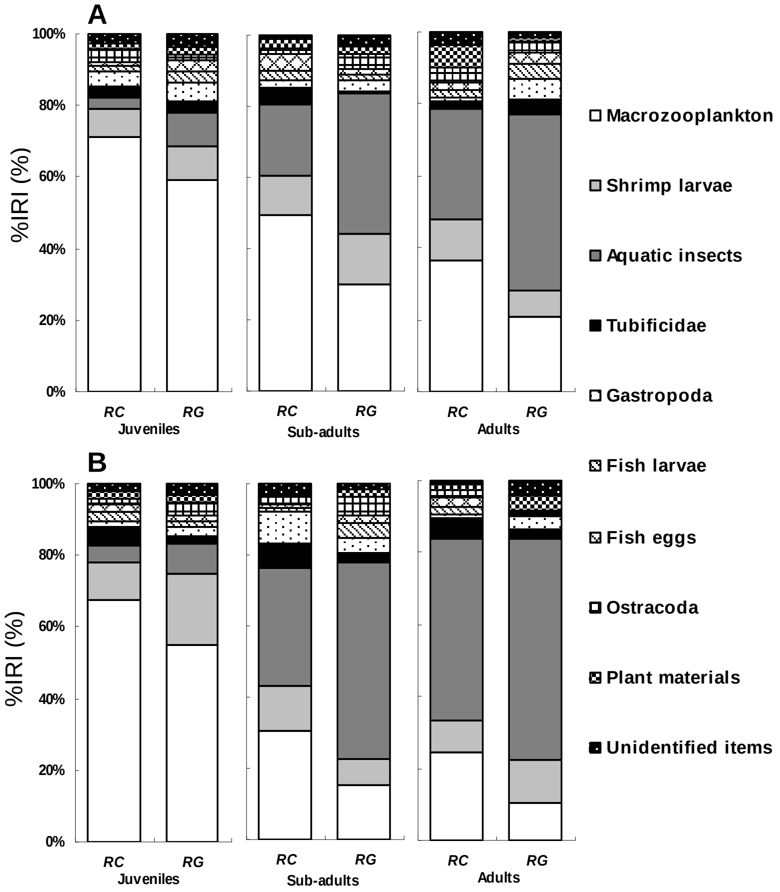

Fig. 3 Diet compositions (%IRI) of *R. cliffordpopei* (*RC*) and *R. giurinus* (*RG*) at three life stages (juveniles, sub-adults and adults) in (A) littoral habitat and (B) profundal habitat of Lake Erhai (China) in 2010.

Table 6 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) of *R. cliffordpopei* and *R. giurinus* between life stages (juveniles, sub-adults and adults) within species in littoral habitat (LH) and profundal habitat (PH) of Lake Erhai (China) in 2010.

			Juveniles			Sub-adults		
			χ^2	d.f.	*P*	χ^2	d.f.	*P*
LH	*R. cliffordpopei*	Sub-adults	21.09	9	**0.012**			
		Adults	38.04	9	<**0.001**	9.98	9	0.352
	R. giurinus	Sub-adults	33.47	9	<**0.001**			
		Adults	47.73	9	<**0.001**	11.59	9	0.237
PH	*R. cliffordpopei*	Sub-adults	33.47	9	<**0.001**			
		Adults	47.72	9	<**0.001**	16.35	9	0.060
	R. giurinus	Sub-adults	66.15	9	<**0.001**			
		Adults	79.73	9	<**0.001**	15.96	9	0.068

The stable isotope analyses (SIA) indicated that the life stages had significant effects on $\delta^{13}C$ and $\delta^{15}N$ (three-way ANOVA, $P < 0.001$, Fig. 4 & Table 7). Both species generally showed a steady decrease in $\delta^{13}C$ and $\delta^{15}N$ from juveniles to adults (Fig. 4). Mixing models revealed that the contribution of macrozooplankton was highest (43.4 - 74.1 %) for juveniles and lowest for adults (4.2 - 7.4 %, Fig. 5). Aquatic insects and Tubificidae contributed little (3.4 - 9.2 %) to the diets of juveniles. They contributed 59.5 - 81.9 % and 89.9 - 90.14 % to the diets of sub-adults and adults, respectively (Fig. 5).

Table 7 Three-way analysis of variances (ANOVA) used to test the differences in stable isotopic values (δ^{13}C and δ^{15}N, the inverse transformed) between life stages (juveniles, sub-adults and adults), species (*R. cliffordpopei* and *R. giurinus*) and habitats (littoral habitat (LH) and profundal habitat (PH)) in Lake Erhai (China) in 2010.

	Sources	SS	d.f.	MS	*F*	*P*
^{13}C (‰)	Life stages	1.11E-3	2	5.57E-4	321.63	<**0.001**
	Species	3.74E-5	1	3.744E-5	21.73	<**0.001**
	Habitats	8.74E-5	1	8.74E-5	50.48	<**0.001**
	Life stages × Species	1.10E-6	2	5.001E-7	0.31	0.730
	Life stages × Habitats	1.16E-6	2	8.00E-7	0.46	0.230
	Species × Habitats	1.25E-5	1	1.25E-7	7.24	**0.008**
	Life stages × Species × Habitats	7.22E-6	2	3.10E-6	2.26	0.111
	Residuals	2.74E-4	158	1.70E-6		
δ^{13}N (‰)	Life stages	4.87E-2	2	2.43E-2	59.43	<**0.001**
	Species	2.97E-3	1	2.97E-3	7.23	**0.008**
	Habitats	0.12	1	0.12	301.98	<**0.001**
	Life stages × Species	5.50E-5	2	2.801E-5	0.67	0.510
	Life stages × Habitats	7.900E-5	2	3.950E-5	1.62	0.204
	Species × Habitats	4.83E-3	1	4.83E-3	11.79	<**0.001**
	Life stages × Species × Habitats	2.40E-4	2	1.20E-4	0.29	0.750
	Residuals	0.06	158	4.10E-4		

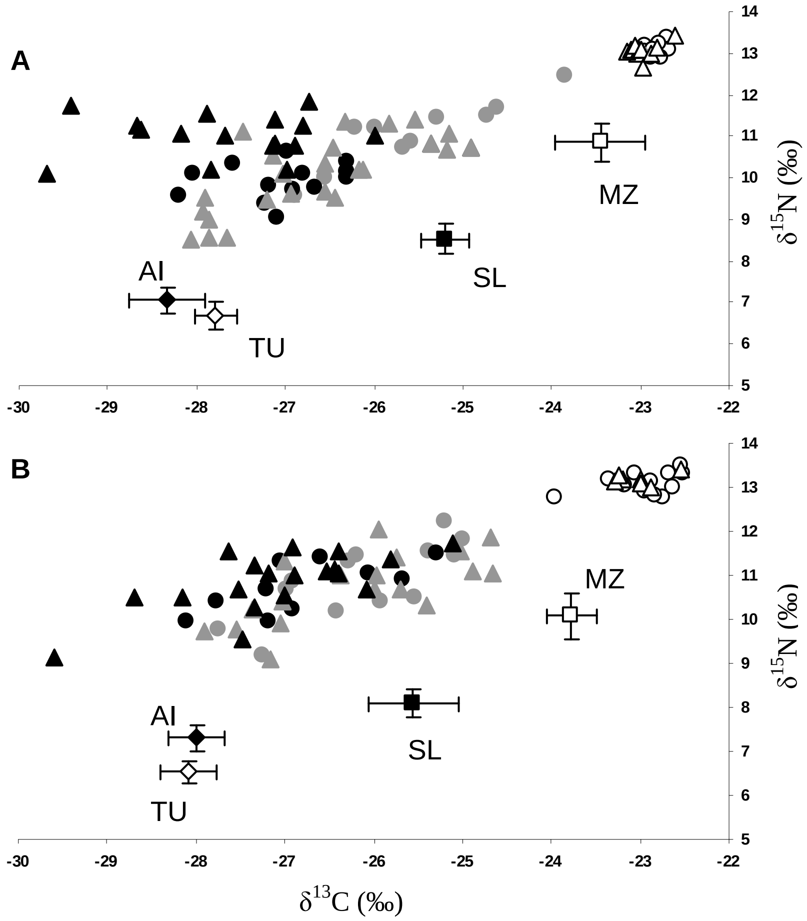

Fig. 4 δ^{13}C (‰) and δ^{15}N (‰) of *R. cliffordpopei* (juveniles (○), sub-adults (●) and adults (●)), *R. giurinus* (juveniles (Δ), sub-adults (▲) and adults (▲)), and their potential food resources (mean ± standard deviation, n=3) in littoral habitat (A) and profundal habitat (B) of Lake Erhai (China) in 2010. The potential food sources were macrozooplankton (MZ), shrimp larvae (SL), aquatic insects (AI) and Tubificidae (TU).

3.2.2 Trophic niche differences between species

The two gobies displayed clear trophic niche differences (i.e. food partitioning) in LH for sub-adult and adult life stage.

In LH and PH, GCA demonstrated that diet compositions (%IRI) of the two species were similar at juveniles (χ^2 test, $P > 0.05$) but significantly different for sub-adults and adults (χ^2 test, $P < 0.05$, Table 8 and Fig. 3). Sub-adults and adults of *R. cliffordpopei* preyed more macrozooplankton than *R. giurinus*, while *R. giurinus* consumed more aquatic insects than *R. cliffordpopei* in the two habitats (Fig. 3).

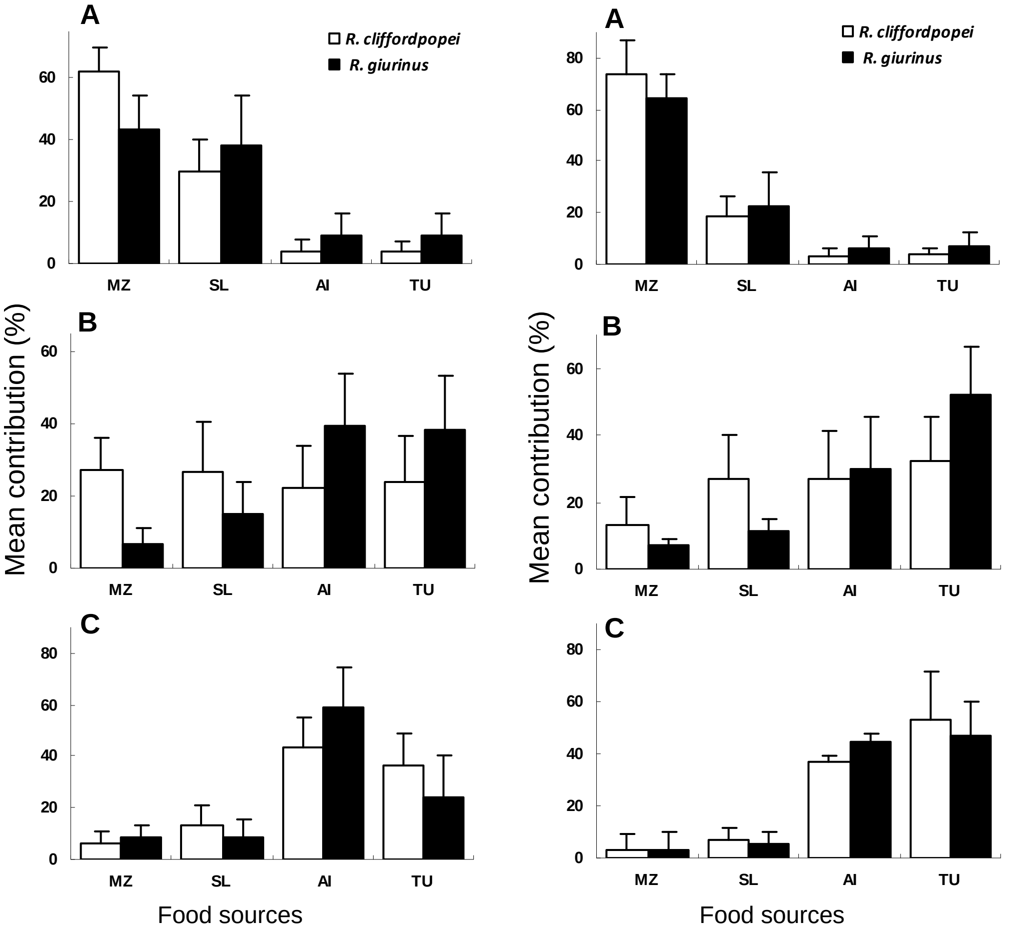

Fig. 5 The contributions (mean ± SD, %) of potential food sources to diets of *R. cliffordpopei* and *R. giurinus* at three life stages (A: juveniles, B: sub-adults and C: adults) in (left) littoral habitat and (right) profundal habitat of Lake Erhai (China) in 2010. The values were calculated using stable isotope mixing models. Potential food resources were macrozooplankton (MZ), shrimp larvae (SL), aquatic insects (AI) and Tubificidae (TU). Bars were standard deviation of each value.

Table 8 Chi-square test (χ^2 test) used to test differences in food compositions (%IRI) between *R. cliffordpopei* and *R. giurinus* within habitat (littoral habitat (LH) and profundal habitat (PH)) at the same life stages (juveniles, sub-adults and adults) in Lake Erhai (China) in 2010.

		R. giurinus								
		Juveniles			Sub-adults			Adults		
	R. cliffordpopei	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
LH	Juveniles	6.07	9	0.732						
	Sub-adults				19.01	9	**0.025**			
	Adults							18.19	9	**0.032**
PH	Juveniles	8.10	9	0.523						
	Sub-adults				22.03	9	**0.008**			
	Adults							19.19	9	**0.024**

Species significantly affected δ^{13}C and δ^{15}N of these two species (three-way ANOVA, $P < 0.05$, Fig. 4 & Table 7). In LH, δ^{13}C and δ^{15}N of juveniles were similar between the two species (Mann-Whitney, $P > 0.05$), but sub-adults of *R. cliffordpopei* showed more enriched δ^{13}C and δ^{15}N values than *R. giurinus*. Adults of *R. cliffordpopei* were more depleted in δ^{15}C but enriched in δ^{15}N compared to *R. giurinus* (Mann-Whitney, $P < 0.05$, Fig. 4 & Table 9). Macrozooplankton and shrimp larvae contributed more to the diets of sub-adults in *R. cliffordpopei* in LH, whereas aquatic insects and Tubificidae contributed more to the diets of sub-adults and adults of *R. giurinus* (Fig. 5). In PH, the two species showed similar δ^{13}C and δ^{15}N for each life stage (Mann-Whitney, $P > 0.05$, Fig. 4 and Fig. 5 & Table 9).

Table 9 Mann-Whitney tests used to test the differences in stable isotopic values (δ^{13}C and δ^{15}N) between *R. cliffordpopei* and *R. giurinus* within habitat (littoral habitat (LH) and profundal habitat (PH)) at the same life stages (juveniles, sub-adults and adults) in Lake Erhai (China) in 2010.

			R. giurinus					
Habitat	Stable isotope		Juveniles		Sub-adults		Adults	
		R. cliffordpopei	W	*P*	W	*P*	W	*P*
LH	δ^{13}C (‰)	Juveniles	117	0.289				
		Sub-adults			155	<0.001		
		Adults					45	0.010
	δ^{13}N (‰)	Juveniles	119	0.239				
		Sub-adults			144	0.003		
		Adults					48	0.015
PH	δ^{13}C (‰)	Juveniles	92	0.640				
		Sub-adults			52	0.341		
		Adults					174	0.229
	δ^{13}N (‰)	Juveniles	93	0.634				
		Sub-adults			69	0.435		
		Adults					98	0.353

3.3 Niche separation along temporal axis

3.3.1 Differences in ages and cohort structures

These two gobies showed similar maximal ages and similar cohort structures but their similar-sized cohorts appeared at different time. There is a temporal partitioning in terms of the appearance of similar-sized cohorts between them. Most of the scales examined were readable for annuli (72.1% for *R. cliffordpopei* and 75.8 % for *R. giurinus*). The maximal ages of the two species are. 0+ based on all these read scales (Fig. 6).

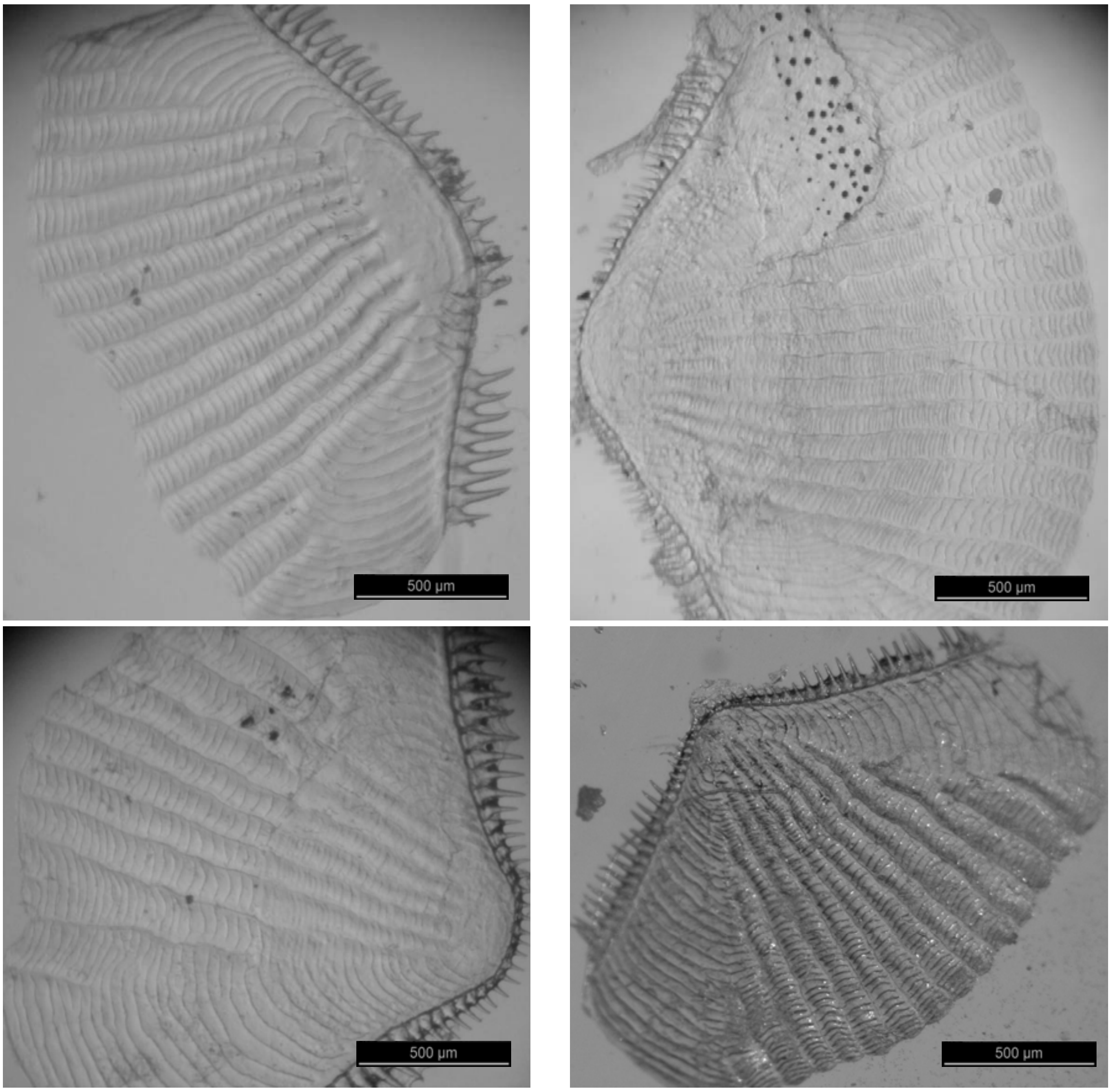

Fig. 6 The scales of *R. giurinus* in November 2009 (LT was 27.1 mm), March 2011 (L_T was 43.3 mm), June 2011 (LT was 35.8 mm) and August 2011 (LT was 56.7 mm) in Lake Erhai (China).

Over the two years study period, a total of 14,462 individuals of *R. cliffordpopei* and 16,793 individuals of *R. giurinus* (Fig. 7) were measured from October 2009 to October 2011. From November to May, 3,935 individuals were identified in sex for *R. cliffordpopei* (2,296 males and 1,639 females, Fig. 8). From January to July, 3,297 individuals were were identified in sex for *R. giurinus* (1,690 males and 1,607 females, Fig. 9).

For *R. cliffordpopei*, the cohorts born in 2009, 2010 and 2011 were found from October 2009 to May 2010, April 2010 to June 2011, and April 201l to October 2011, respectively (Fig. 7). In *R. giurinus*, the cohorts born in 2009, 2010 and 2011 were found from October 2009 to July 2010, July 2010 to August 2011, and July 201l to October 2011, respectively (Fig. 7). Body length-frequency between the two species was significantly different from December 2009 to October 2010 and from April to October 2011(K-S test, $P < 0.001$, Fig. 7 & Table 10). For males and females of *R. giurinus*, there was a single cohort from January to July in 2010 (the cohort was born in 2009) and another one in 2011 (the cohort was born in 2010, Fig. 8). Males and females showed significantly different body length-frequency in December 2009, January and April 2010, November and March 2011 (K-S test, $P < 0.05$, Table 10). Similarly, both sexes of *R. giurinus* showed a single cohort from January to July in 2010 and another one 2011 (Fig. 9). They displayed similar body length-frequency between sexes except April 2010 (K-S test, $P > 0.05$, Table 10).

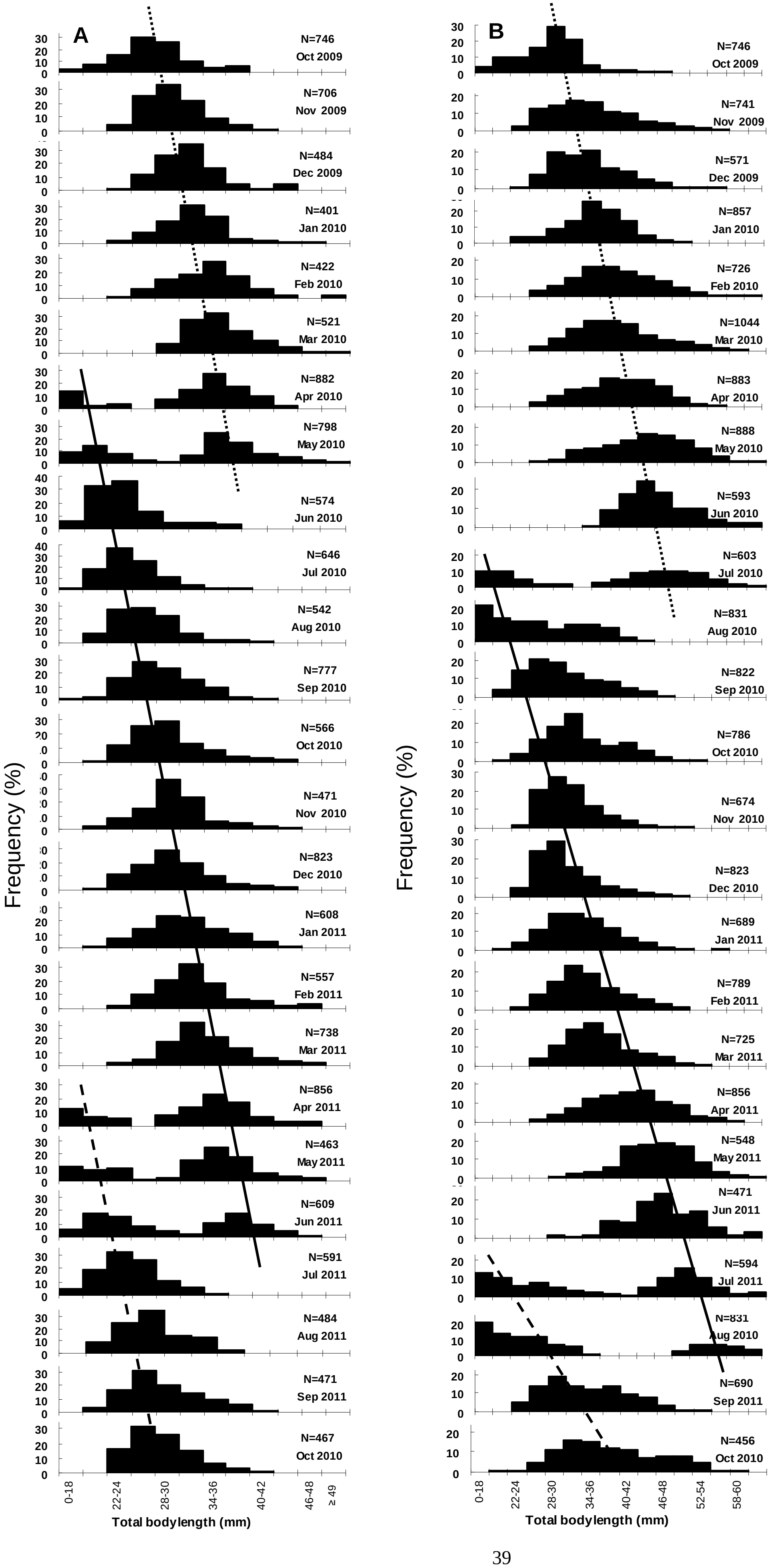

Fig. 7 Comparison of th body length-frequencies for the entire populations of (A) *R. cliffordpopei* and (B) *R. giurinus* from October 2009 to October 2011 in Lake Erhai (China). The dotted lines connect the cohort born in 2009. The solid lines connect the cohort born in 2010. The dashed lines connect the cohort born in 2011.

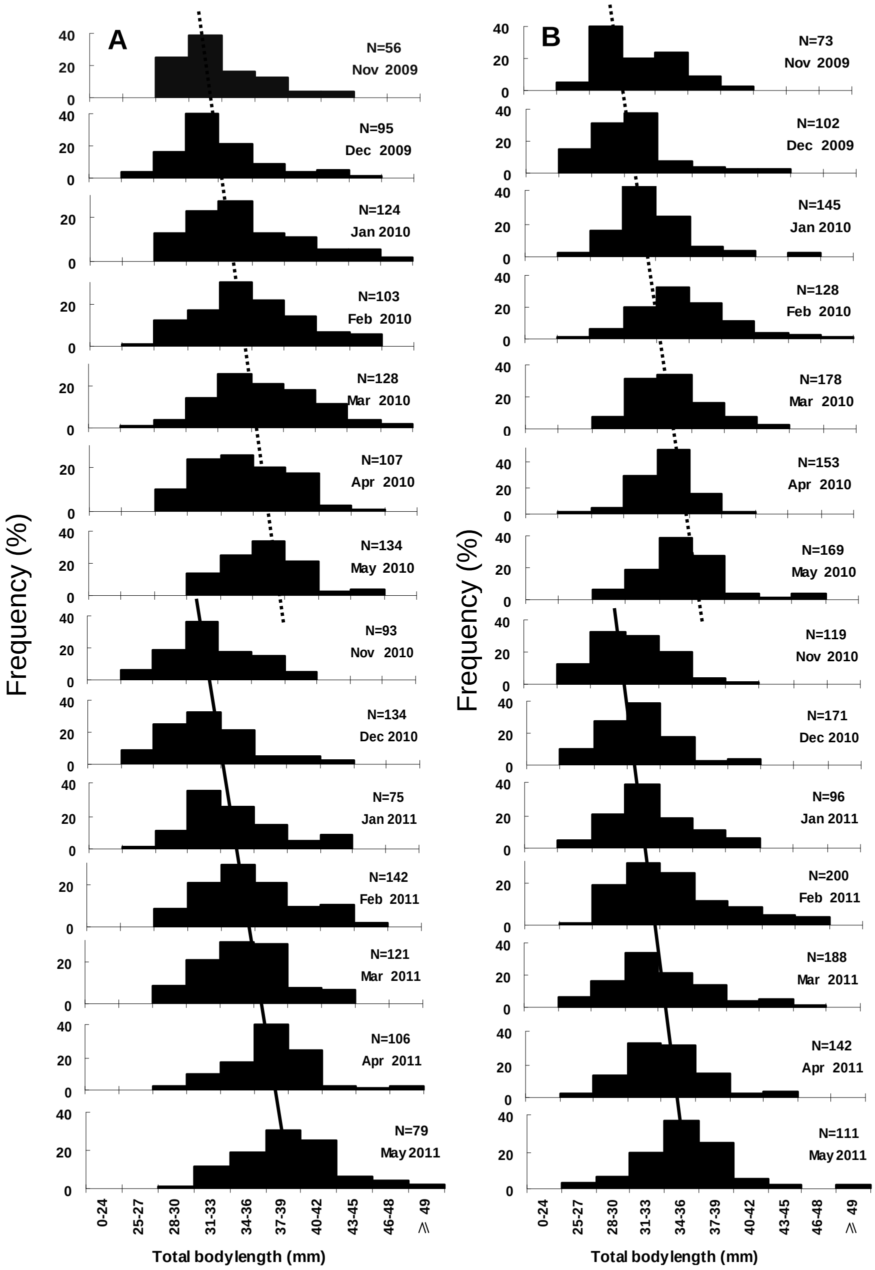

Fig. 8 Comparison of the body length-frequencies for (A) males and (B) females of *R. cliffordpopei* from November 2009 to May 2011 in Lake Erhai (China). The dotted line connects the cohort born in 2009. The solid line connects the cohort born in 2010.

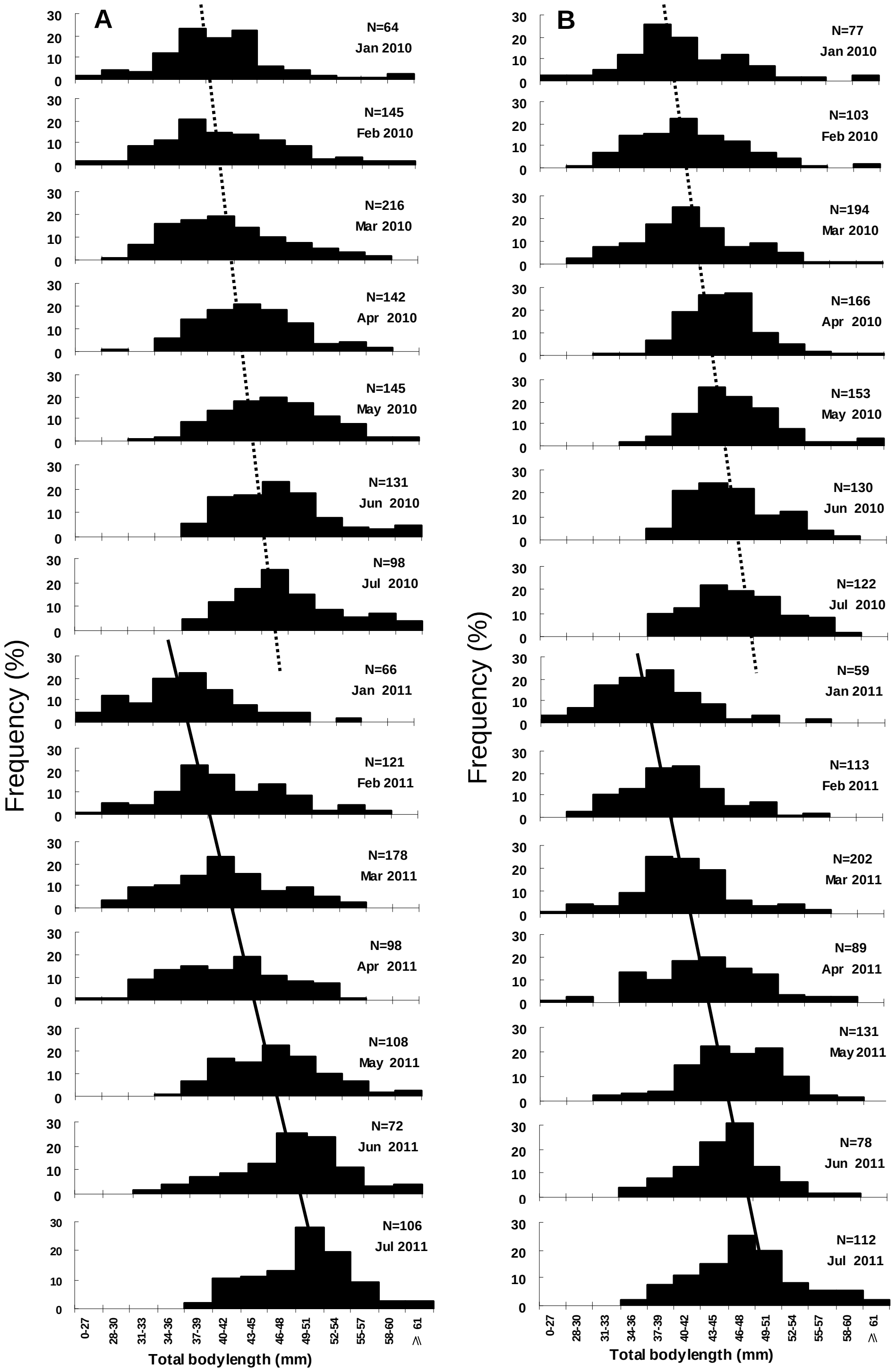

Fig. 9 Comparison of the body length-frequencies for (A) males and (B) femalesof *R. giurinus* from January 2010 to July 2011 in Lake Erhai (China). The dotted line connects the cohort born in 2009. The solid line connects the cohort born in 2010.

Table 10 Kolmogorov-Smirnov (K-S) test used to test differences in body length-frequency distributions of *R. cliffordpopei* and *R. giurinus* between sexes within species and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		D	*P*	D	*P*	D	*P*
2009	Oct					0.149	0.126
	Nov	0.094	0.084			0.198	0.005
	Dec	0.311	<0.001			0.308	<0.001
2010	Jan	0.276	<0.001	0.095	0.779	0.181	<0.001
	Feb	0.138	0.153	0.079	0.836	0.098	0.009
	Mar	0.102	0.115	0.073	0.646	0.272	<0.001
	Apr	0.277	<0.001	0.158	0.043	0.533	<0.001
	May	0.071	0.212	0.115	0.275	0.614	<0.001
	Jun			0.103	0.486	0.949	<0.001
	Jul					0.534	<0.001
	Aug					0.363	<0.001
	Sep					0.183	<0.001
	Oct					0.373	<0.001
	Nov	0.276	<0.001			0.107	0.314
	Dec	0.091	0.172			0.098	0.896
2011	Jan	0.116	0.207	0.067	0.532	0.120	0.140
	Feb	0.097	0.140	0.101	0.579	0.057	0.409
	Mar	0.209	<0.001	0.117	0.604	0.114	0.143
	Apr	0.114	0.106	0.125	0.089	0.332	<0.001
	May	0.082	0.331	0.108	0.112	0.491	<0.001
	Jun			0.088	0.321	0.788	<0.001
	Jul					0.654	<0.001
	Aug					0.341	<0.001
	Sep					0.197	<0.001
	Oct					0.294	<0.001

3.3.2 Differences in growth patterns

Growth patterns of the two species were different based on mean total length, body condition and Electronic Length Frequency Analysis. In *R. cliffordpopei*, mean total body length (TL) of males and females steadily increased from November to

February but there was almost no increase for females from March to May (Fig. 10). The males showed significantly higher TL than females except in February, November and December 2010 (Mann-Whitney test, $P < 0.05$, Table 11). Both sexes of *R. giurinus* showed a similar growth in TL from January to July (Mann-Whitney test, $P > 0.05$, Fig. 10 & Table 11). For the entire populations, TL of *R. cliffordpopei* peaked in March and dropped to the lowest values in July, while *R. giurinus* showed the highest TL in June and the lowest one in August (Fig. 10). Their TL differed significantly in November 2009, January to July except March 2010, and April to October except August 2011 (Mann-Whitney test, $P < 0.05$, Table 11).

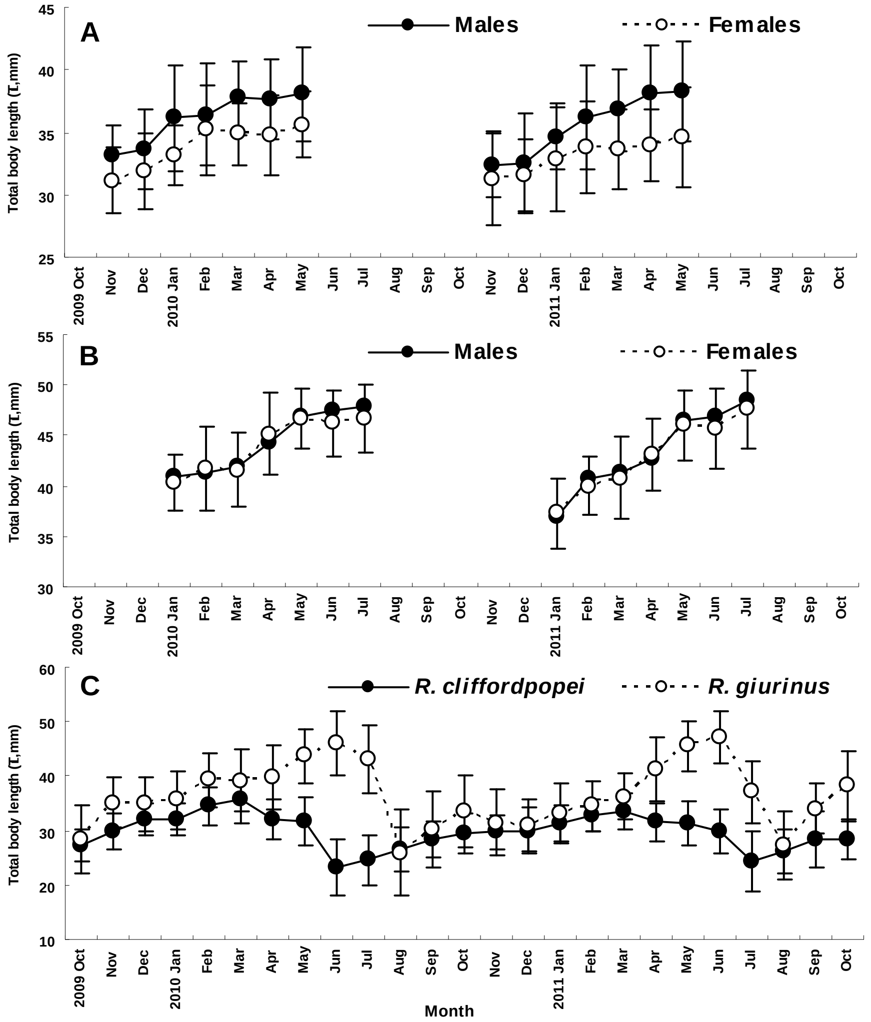

Fig. 10 Comparison of monthly changes in body length (mm) for males and females of (A) *R. cliffordpopei* and (B) *R. giurinus*, and (C) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (A) and (B) indicated that individuals unidentifiable for sexes.

Table 11 Mann-Whitney test used to test differences in total body length (mm) of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		W	*P*	W	*P*	W	*P*
2009	Oct					14383	0.156
	Nov	29307	<0.001			71963	<0.001
	Dec	34881	<0.001			15435	0.089
2010	Jan	25143	<0.001	50941	0.687	14577	0.008
	Feb	10657	0.177	74114	0.847	19020	<0.001
	Mar	24571	0.001	21184	0.846	24578	0.104
	Apr	17451	<0.001	10543	0.110	65879	<0.001
	May	20167	<0.001	11659	0.446	54864	<0.001
	Jun			93141	0.189	12812	<0.001
	Jul					65815	<0.001
	Aug					79154	0.205
	Sep					30555	0.123
	Oct					45411	0.012
	Nov	14572	0.102			25827	0.156
	Dec	10294	0.114			37188	0.475
2011	Jan	24394	0.009	11274	0.501	27154	0.318
	Feb	24786	<0.001	20560	0.432	34182	0.167
	Mar	50149	<0.001	31476	0.389	17457	0.209
	Apr	43744	<0.001	28744	0.517	34715	<0.001
	May	54784	<0.001	76981	0.240	54724	<0.001
	Jun			34157	0.177	34781	<0.001
	Jul			14958	0.108	19785	<0.001
	Aug					45784	0.341
	Sep					24516	<0.001
	Oct					31254	<0.001

Body condition of females and males of *R. cliffordpopei* increased in January and subsequently dropped in May (Fig. 11), whereas body condition of *R. giurinus* were highest in May and lowest in July (Fig. 11). Females showed significantly higher body condition than males from January to April for *R. cliffordpopei*, and from March to June for *R. cliffordpopei* (Mann-Whitney test, $P < 0.05$, Table 12).

The entire populations of *R. cliffordpopei* had higher body condition than *R. giurinus* in October 2009, January to February to July 2010, and February to March 2011 (Mann-Whitney test, $P < 0.05$, Fig. 11 & Table 12).

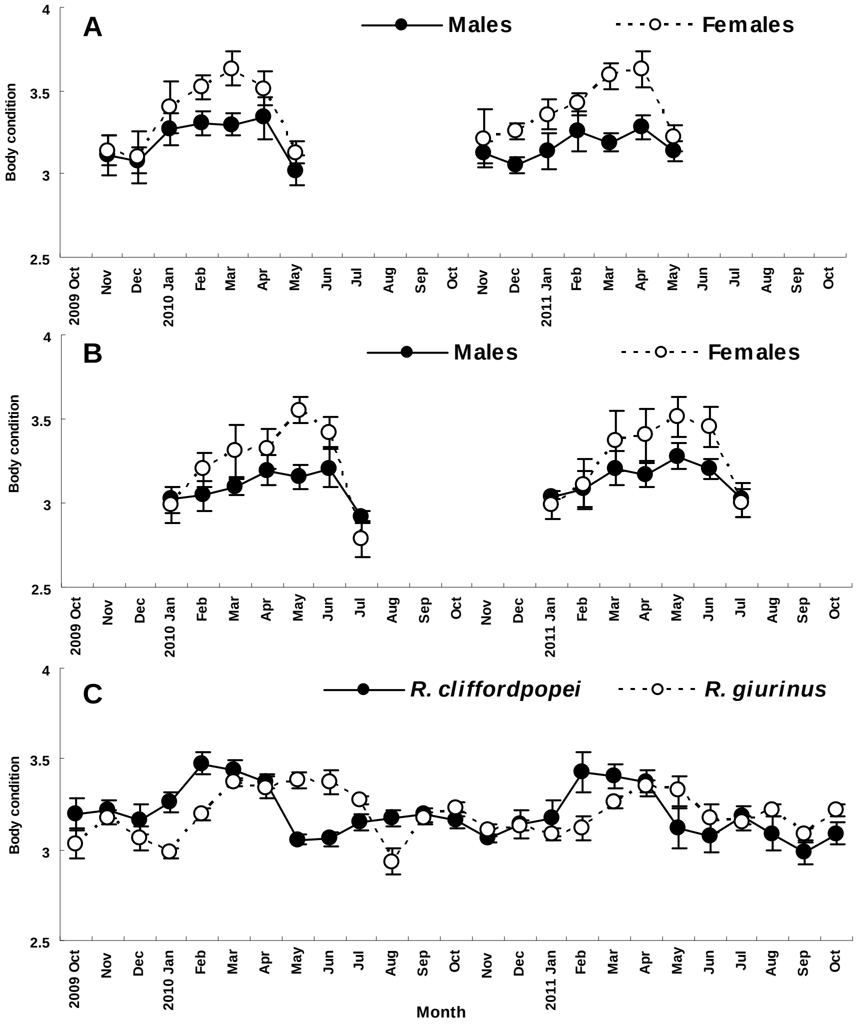

Fig. 11 Comparison of monthly changes in body condition females and females of (A) *R. cliffordpopei* and (B) *R. giurinus*, and (C) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (a) and (b) indicated that individuals unidentifiable for sexes.

Table 12 Analyses of covariance (ANCOVAs, total body length is a covariate) used to test the differences in body condition of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011. The *F* and *P* values are effects of sexes on body condition within species, and effects of species on body condition for the entire populations.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		F	*P*	*F*	*P*	*F*	*P*
2009	Oct					16.771	<0.001
	Nov	0.907	0.343			1.797	0.184
	Dec	2.608	0.111			4.615	0.035
2010	Jan	3.140	0.089	2.2305	0.139	88.442	<0.001
	Feb	105.124	<0.001	10.104	0.002	53.264	<0.001
	Mar	122.830	<0.001	41.508	<0.001	2.3367	0.129
	Apr	6.118	0.016	20.342	<0.001	0.868	0.354
	May	6.515	0.012	89.543	<0.001	93.635	<0.001
	Jun			31.875	<0.001	77.237	<0.001
	Jul			11.678	0.001	7.569	0.007
	Aug					37.455	<0.001
	Sep					0.382	0.5381
	Oct					2.981	0.087
	Nov	3.461	0.066			1.379	0.243
	Dec	24.563	<0.001			1.936	0.167
2011	Jan	19.876	<0.001	2.804	0.098	0.531	0.467
	Feb	9.211	0.003	1.871	0.175	50.212	<0.001
	Mar	18.982	<0.001	44.630	<0.001	7.390	0.008
	Apr	145.622	<0.001	79.381	<0.001	0.352	0.555
	May	1.954	0.161	27.564	<0.001	24.213	<0.001
	Jun			34.070	<0.001	7.092	0.009
	Jul			2.329	0.131	3.558	0.063
	Aug					4.759	0.0319
	Sep					2.878	0.093
	Oct					6.021	0.016

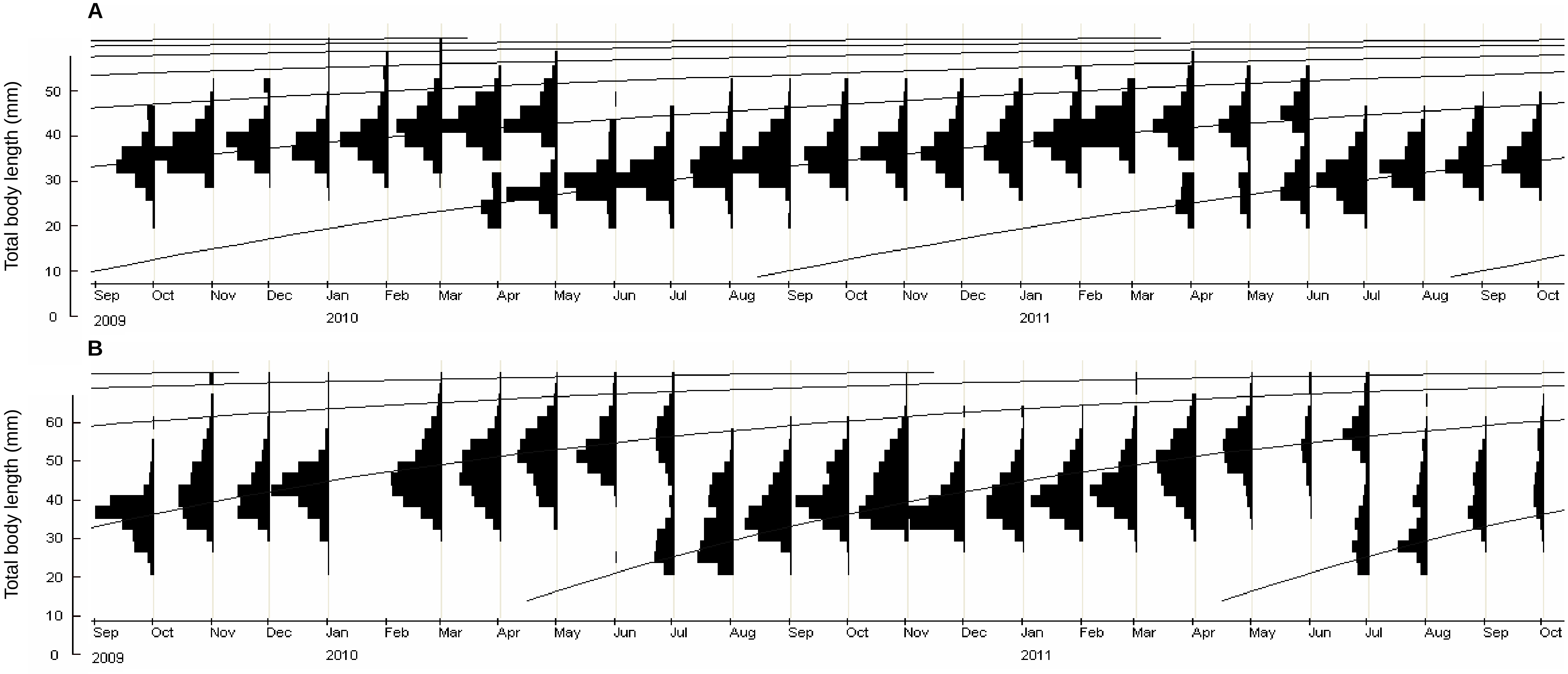

Fig. 12 Growth lines of (A) *R. cliffordpopei* and (B) *R. giurinus* using Electronic Length Frequency Analysis (ELEFAN). The von Bertalanffy growth function by ELEFAN was $L_t = 55.65\,[- e^{-0.58\,(t + 1.48)}]$ (Score = 0.477) for *R. cliffordpopei* and $L_t = 68.25\,[- e^{-0.99\,(t + 0.78)}]$ (Score = 0.216), respectively.

Electronic Length Frequency Analysis (ELEFAN) demonstrated that the asymptotic length (L_∞) was 55.65 mm for *R. cliffordpopei* and 68.25 mm for *R. giurinus*. The growth coefficient (K) was 0.58 for *R. cliffordpopei* and 0.99 mm for *R. giurinus*. The von Bertalanffy growth function was L_t = 55.65 [- $e^{-0.58\,(t\,+\,1.48)}$] (Score = 0.477) for *R. cliffordpopei* and L_t = 68.25 [- $e^{-0.99\,(t\,+\,0.78)}$] (Score = 0.216, Fig. 12), respectively.

3.3.3 Differences in reproduction cycles

Rhinogobius cliffordpopei and *R. giurinus* showed different onsets of spawning and different peaks of spawning seasons. *R. cliffordpopei* spawned from February to June, with a spawning peak occurring in March and April. Spawning activity of *R. giurinus* occurred from April to August with a peak activity during May and June. Specifically, the maturity stage of most ovaries and testes of *R. cliffordpopei* were at stage-I from July to October, and the sex of individuals was unidentifiable during this period. Individuals with stage-IV ovaries appeared first in February. The proportions of stage-IV ovaries were higher than 90 % in March and April, and decreased steadily from May to June (Fig. 13). The gonado-somatic indices (I_G) of females increased greatly in February and showed the highest values in March and April (Fig. 14). Mean I_G of females was 18.8 % during the peak spawning seasons (March to April). For *R. giurinus*, gonads of most individuals were at stage-I from September to December. Mature individuals (these with stage-IV ovaries) were first observed in April and accounted for 76-86 % in May and June (Fig. 13). I_G of females increased significantly in April, peaked in June and decreased in July (Fig. 14). Mean I_G of females during the peak spawning seasons was 12.4 % (May to June).

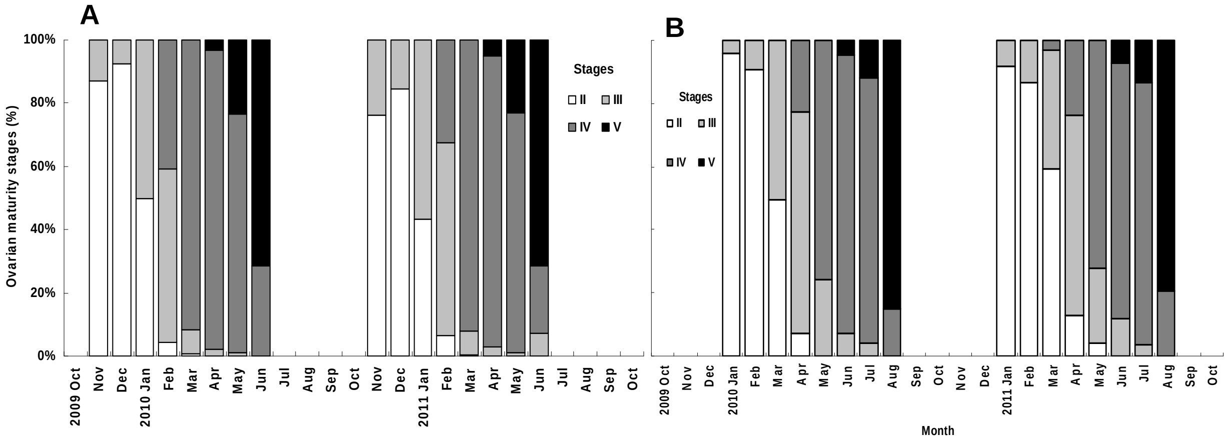

Fig. 13 Monthly changes in the percentage of ovarian maturity stages for (A) *R. cliffordpopei* and (B) *R. giurinus* from October 2009 to October 2011 in Lake Erhai (China). The absence of data indicated that individuals unidentifiable for sexes.

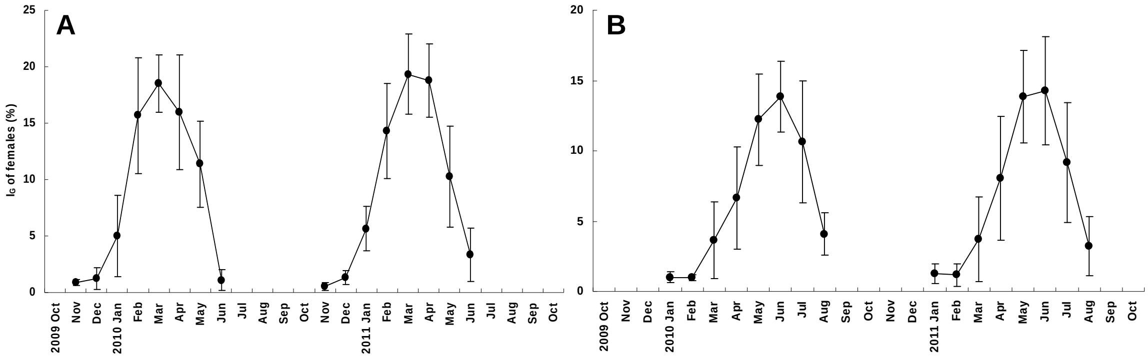

Fig. 14 Monthly changes in the gonado-somatic indices (I_G) of females for (A) *R. cliffordpopei* and (B) *R. giurinus* from Oct 2009 to Oct 2011 in Lake Erhai (China). The absence of data indicated that individuals unidentifiable for sexes. The error-bars represent the standard deviations.

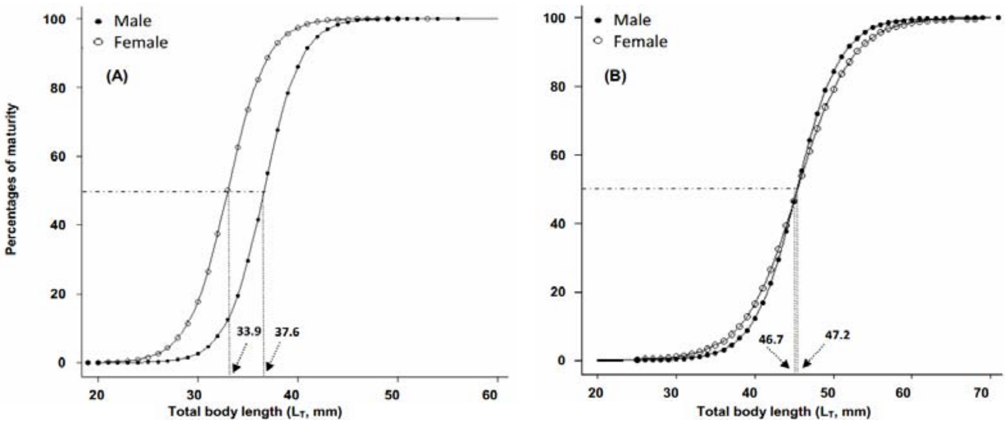

Fig. 15 Comparison of the size of maturity of (A) *R. cliffordpopei* (n = 2293 for females and n = 1819 for males) and (B) *R. giurinus* (n = 2237 for females and n = 2304 for males) from October 2009 to October 2011 in Lake Erhai (China). Mean size of maturity (L_{m50}) was 33.9 mm and 47.2 mm for females and 37.6 mm and 46.7 mm for males of *R. cliffordpopei* and *R. giurinus*.

The mean size of maturity (L_{m50}) of males in *R. cliffordpopei* was 37.6 mm, which was significantly larger than 33.9 mm for females (Likelihood ratio test, df = 2, Chi-squared = 259.82, $P < 0.001$). *R. giurinus* showed similar L_{m50} between males (46.7 mm) and females (47.2 mm, Likelihood ratio test, df = 2, Chi-squared = 4.62, $P = 0.099$) (Fig. 15).

3.3.4 Differences in population dynamics

R. cliffordpopei showed higher total mortality ($Z = 2.12$), fishing mortality ($F = 1.25$), exploitation rate ($E = 0.59$) than *R. giurinus* ($Z = 1.73$, $F = 0.56$, $E = 0.32$, respectively), whereas the natural mortality of *R. cliffordpopei* ($M = 0.85$) was lower than of *R. giurinus* ($M = 1.17$, Fig. 16).

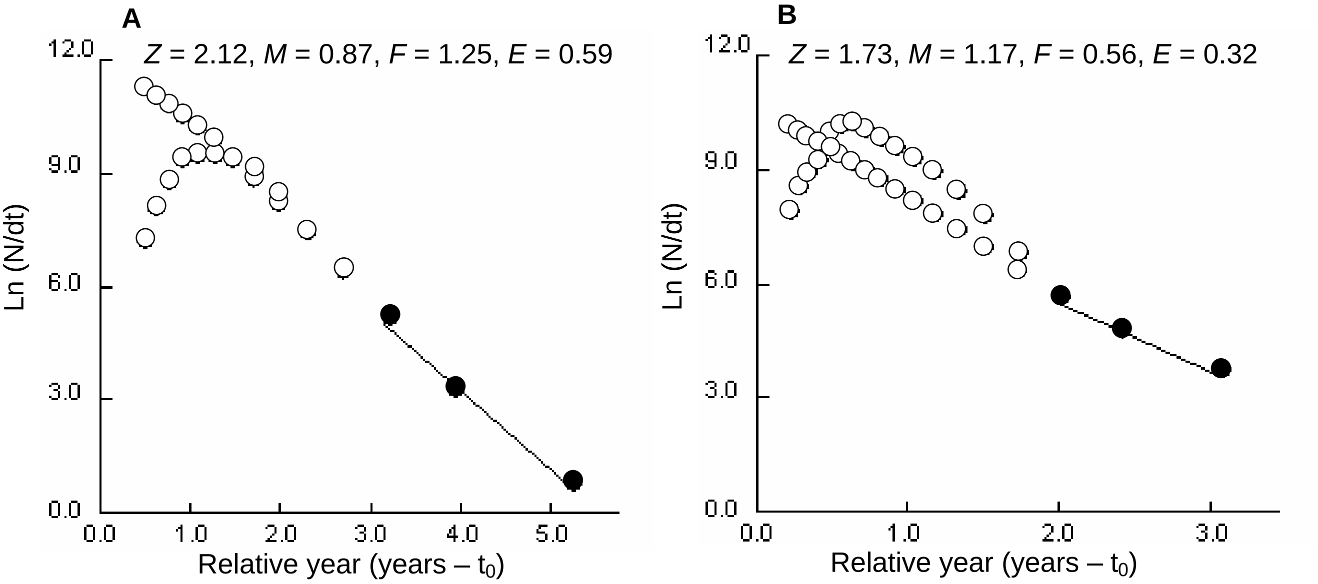

Fig. 16 Body length converted catch curve and mortality estimates for (A) *R. cliffordpopei* and (B) *R. giurinus* in Lake Erhai (China) from October 2009 to October 2011. The estimations were conducted using the parameters of the von Bertalanffy growth function and mean temperature of 18.7°C. *Z*, total mortality; *M*, natural mortality; *F*, fishing mortality; *E*, exploitation rate. The regression was fitted using ● data points on the curve.

The catch per unit effort (CPUE) differed significantly between sexes in *R. cliffordpopei* (RM ANOVAs, $P < 0.001$, Table 13) but insignificantly for *R. giurinus* (RM ANOVAs, $P = 0.122$, Table 13). The females of *R. cliffordpopei* showed significantly higher CPUE than males in November 2009, February to May 2010 and 2011 (Mann-Whitney test, $P < 0.05$, Fig. 17 & Table 14).

The CPUE of the entire populations were significantly different between species

(RM ANOVAs, $P < 0.001$, Table 13). *Rhinogobius cliffordpopei* displayed higher CPUE than *R. giurinus* in July to August 2010, July to September 2011, whereas *R. giurinus* showed higher CPUE than *R. cliffordpopei* in April to June and October to December 2010, and April to June 2011 (Mann-Whitney test, $P < 0.05$, Fig. 17 & Table 14). The CPUE of the populations was low from April to June in *R. cliffordpopei* and from July to September in *R. giurinus* (Fig. 17).

Table 13 Repeated-measures ANOVAs (RM-ANOVA) used to test differences in cath per unit efforts (CPUE, log-transformed) of *R. giurinus* and *R. cliffordpopei* between sexes (fixed factor) within species, and between species (fixed factor) for the entire populations with months as random factors in Lake Erhai (China) from October 2009 to October 2011.

	Sources	SS	d. f.	MS	*F*	*P*
R. cliffordpopei	Sexes	3.658	1	3.658	55.372	<0.001
	Error	1.454	22	0.066		
	Months	50.175	3.411	14.710	52.106	<0.001
	Error	21.184	75.040	0.028		
	Sexes × Months	5.282	3.549	1.488	3.406	0.014
R. giurinus	Sexes	0.267	1	0.267	2.508	0.122
	Error	4.043	38	0.106		
	Months	170.028	3.549	47.913	109.653	<0.001
	Error	58.923	134.849	0.437		
	Sexes × Months	2.190	3.411	0.642	2.274	0.079
The entire populations	Species	5.800	1	5.800	74.549	<0.001
	Error	2.334	30	0.078		
	Months	75.730	6.445	11.750	29.892	<0.001
	Error	76.004	193.352	0.393		
	Species × Months	83.056	8.671	9.579	32.784	<0.001

Table 14 Mann-Whitney test used to test differences in catch per unit effort (CPUE) of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		W	*P*	W	*P*	W	*P*
2009	Oct					240	<0.001
	Nov	21	0.003			89	0.239
	Dec	47	0.148			128	0.754
2010	Jan	57	0.399	172	0.456	147	0.293
	Feb	18	0.002	155	0.2267	41	0.002
	Mar	9	<0.001	131	0.073	112	0.105
	Apr	15	0.001	184	0.533	44	0.003
	May	27	0.009	117	0.026	215	<0.001
	Jun			135	0.081	254	<0.001
	Jul			152	0.199	233	<0.001
	Aug					200	<0.001
	Sep					109	0.097
	Oct	160	0.288			47	<0.001
	Nov	40	0.071			214	<0.001
	Dec	45	0.105			35	<0.001
2011	Jan	51	0.218	188	0.755	202	<0.001
	Feb	18	0.002	192	0.839	191	0.005
	Mar	12	<0.001	139	0.089	100	0.459
	Apr	30	0.009	144	0.130	128	0.007
	May	15	0.001	162	0.293	228	<0.001
	Jun			149	0.170	178	0.002
	Jul			151	0.101	33	<0.001
	Aug					56	<0.001
	Sep					14	<0.001
	Oct					99	<0.001

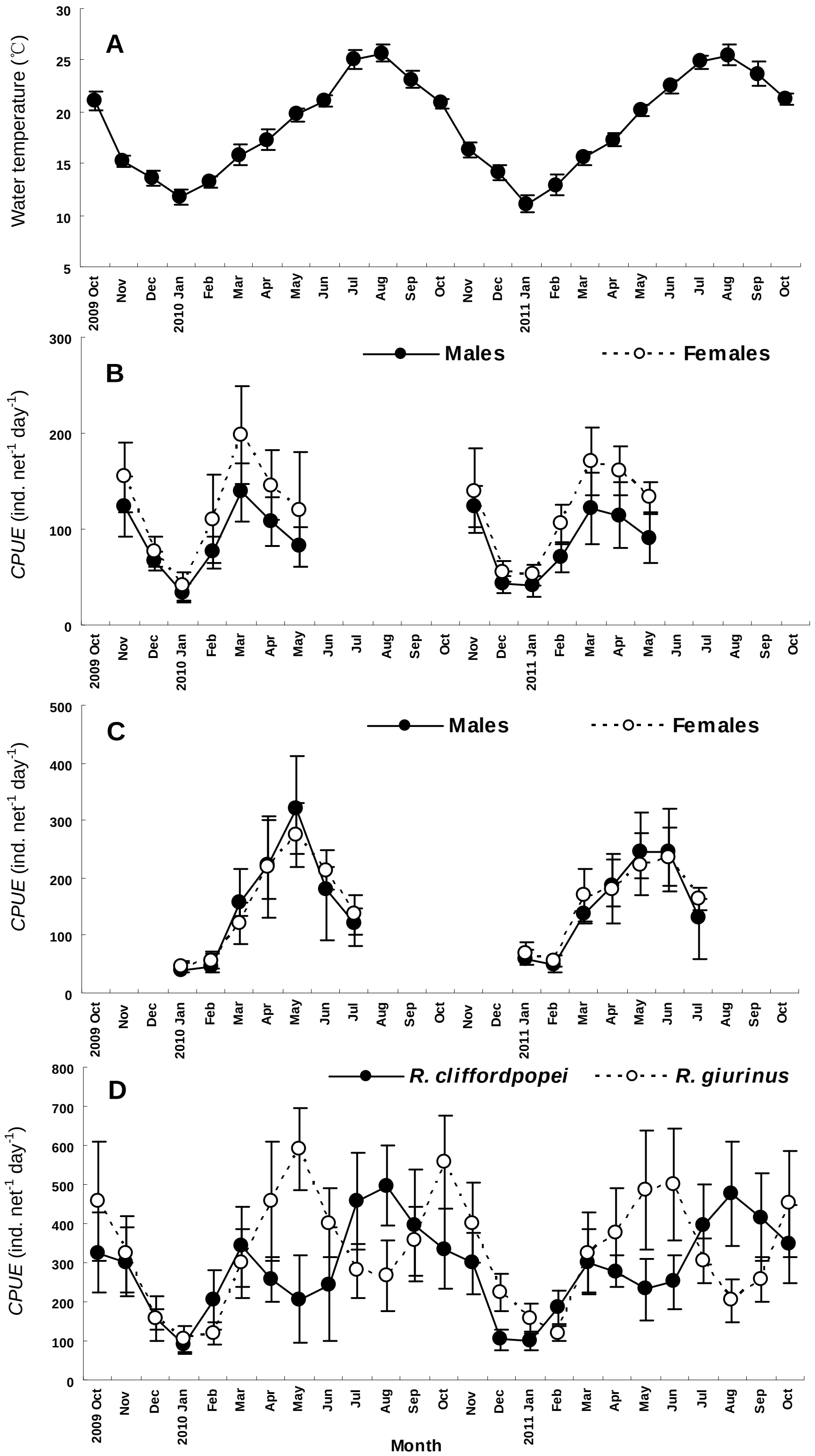

Fig. 17 Monthly changes in (A) water temperature and comparison of catch per unit effort (CPUE) for males and females of (B) *R. cliffordpopei* and (C) *R. giurinus*, and (D) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (B) and (C) indicated that individuals unidentifiable for sexes.

3.3.5 The same diel activity and feeding rhythm

Activity patterns (PCPUE) of the two gobies were similar (Mixed-Effects Models, P = 0.347, Fig. 18 & Table 15) and both of them had two peaks of activity in 24 hours (6:00 - 10:00 and 18:00 - 22:00) in the four seasons (Fig. 18). Although Mixed-Effects Models revealed that seasons had significant effects on PCPUE (Mixed-Effects Models, P <0.001, Table 15), the subsequent one-way ANOVAs suggested that the PCPUE be not significantly different between seasons within species ($F_{3,236}$ = 0.781, P = 0.506 for *R. cliffordpopei* and $F_{3,236}$ = 0.844, P = 0.471 for *R. cliffordpopei*).

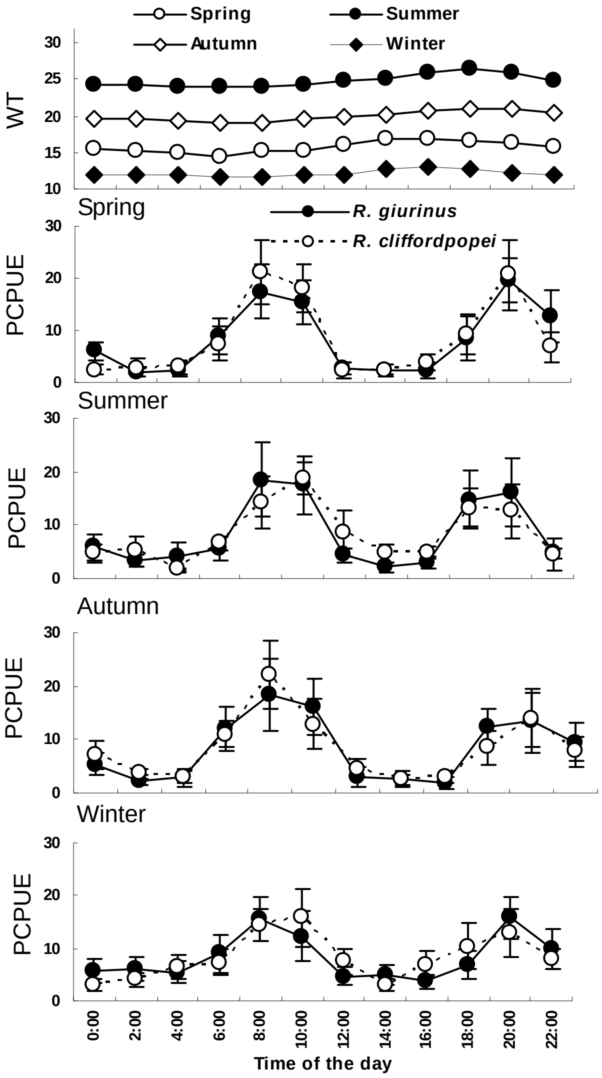

Fig. 18 Diel changes in water temperature (WT, °C) and the percentage of catch per unit effort (PCPUE, %) of *R. giurinus* and *R. cliffordpopei* in the four seasons of 2011 in Lake Erhai (China). Bars are standard deviations.

Table 15 Mixed-Effects Models used to test the differences in the percentage of catch per unit effort (PCPUE, log (x+1) transformed) of *R. giurinus* and *R. cliffordpopei* among seasons and species with seasons and species as fixed factors and the time of a day as a random factor in 2011 in Lake Erhai (China).

Sources	Num. d.f.	Den. d.f.	*F*	*P*
Seasons	3	454	8.179	<0.001
Species	1	454	0.886	0.347
Time	1	10	1.011	0.338
Species × Seasons	3	454	1.077	0.358
Species × Time	1	454	0.588	0.444
Seasons × Time	3	454	7.176	<0.001
Species × Seasons × Time	3	454	2.791	0.040

The two species showed two main feeding peaks were observed per 24 hours, i.e. 6:00 - 10:00 and 18:00 - 22:00 (Fig. 19 & Fig. 20). The feeding activity was similar between the two species but significantly different among seasons within species (Fig. 20 & Table 16). Specifically, they showed similar WGC (Mixed-Effects Models, P = 0.531, Table 16) and PEG (Mann-Whitney test, P = 0.233, 0.410, 0.722 and 0.964 for spring, summer, autumn and winter, Fig. 20). Among seasons, PEG of the two species were significantly lower in winter (Mann-Whitney test, $P < 0.05$), but similar among the three other seasons (Mann-Whitney test, $P > 0.1$, Fig. 20 & Table 17). WGC varied significantly among seasons (Mixed-Effects Models, $P < 0.001$, Table 16). *Rhinogobius giurinus* showed the lowest WGC in winter and highest in spring (Tukey's HSD, $P < 0.001$), but showed similar values in summer and autumn (Tukey's HSD P = 0.863, Fig. 20). For *R. cliffordpopei*, WGC was lowest in winter (Tukey's HSD, $P < 0.001$) and higher in autumn than in spring (Tukey's HSD, P = 0.013), whereas it was similar between spring and summer (Tukey's HSD, P = 0.574), and between summer and autumn (Tukey's HSD, P = 0.238, Fig. 20).

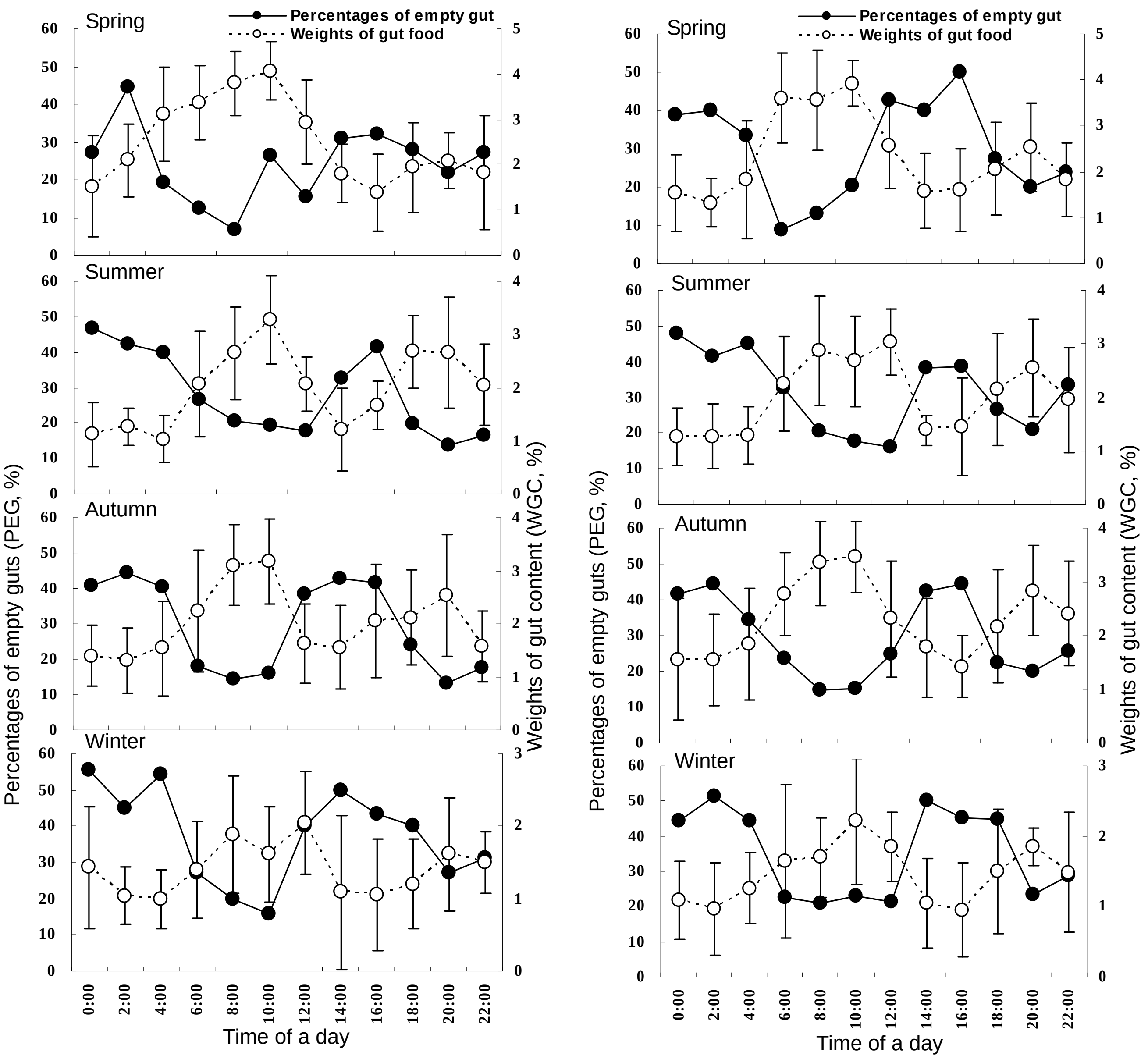

Fig. 19 Diel changes in percentages of empty gut (PEG, %) and weights of gut content (WGC, %) of (left) *R. giurinus* and (right) *R. cliffordpopei* in the four seasons of 2011 in Lake Erhai (China). Bars are standard deviations.

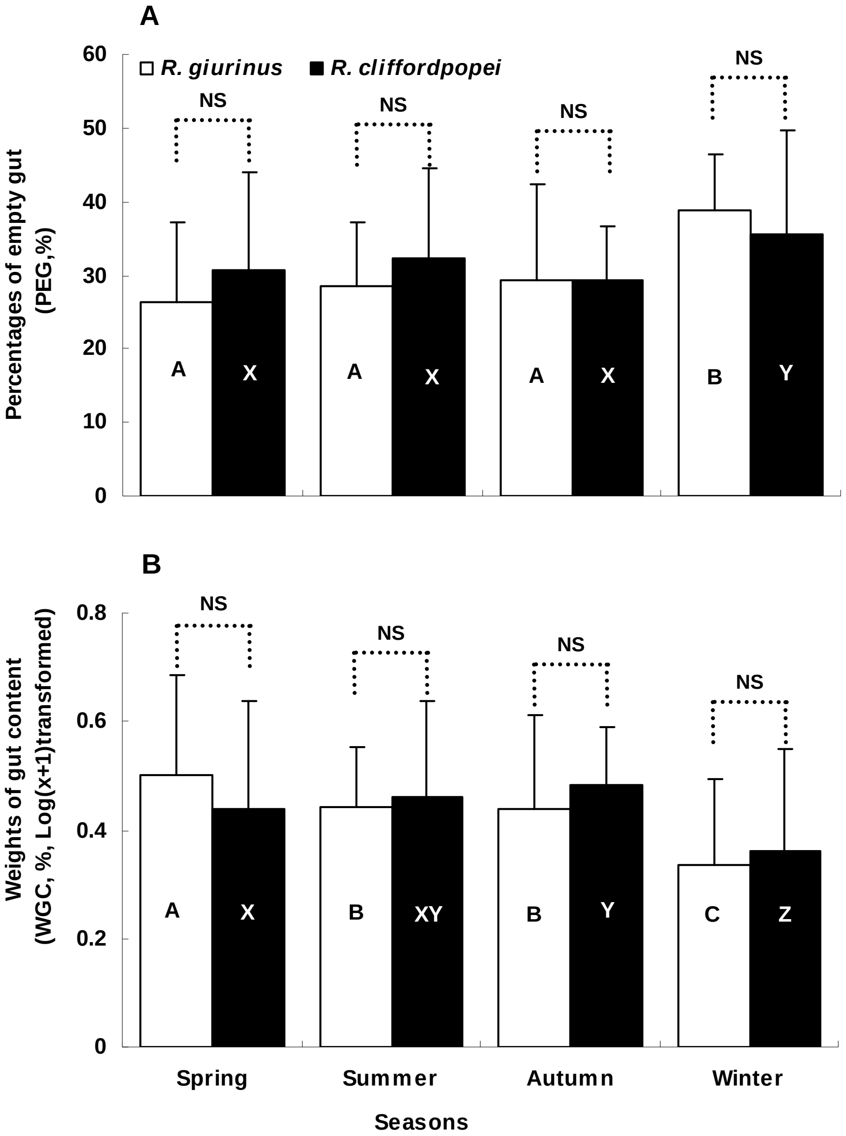

Fig. 20 Seasonal changes (A) in percentages of empty gut (PEG, %) and (B) weights of gut content (WGC, %, log(x+1) transformed) of (left) *R. giurinus* and (right) *R. cliffordpopei* in 2011 in Lake Erhai (China). Bars are standard deviations. The means with different letters in histograms are significantly different among seasons within species. The means with NS are not significantly different between species within the same season

Table 16 Mixed-Effects Models used to test the differences in weights of gut food (WGC, log (x+1) transformed) among seasons and species with seasons and species as fixed factors and the time of a day as a random factor.

Sources	Num. d.f.	Den. d.f.	*F*	*P*
Seasons	3	1495	46.812	<0.001
Species	1	1495	0.392	0.531
Time	1	10	0.656	0.437
Species × Seasons	3	1495	4.517	0.004
Species × Time	1	1495	0.009	0.925
Seasons × Time	3	1495	3.577	0.014
Species × Seasons × Time	3	1495	0.841	0.472

Table 17 Mann-Whitney test used to test the differences in percentages of empty gut (PEG) of *R. giurinus* and *R. cliffordpopei* among seasons within species in 2011 in Lake Erhai (China).

		winter		spring		summer	
		V	*P*	V	*P*	V	*P*
R. giurinus	spring	74	0.003				
	summer	65	0.042	36	0.850		
	autumn	77	<0.001	23	0.398	35	0.791
R. cliffordpopei	spring	65	0.042				
	summer	66	0.034	31	0.569		
	autumn	72	0.007	35	0.893	54	0.266

4 Discussion

4.1 Niche separation along spatial axis

The present study demonstrated that the two congeneric and introduced freshwater goby species display a strong level of habitat segregation (i.e. niche separation along spatial axis) in Lake Erhai. *R. giurinus* mostly occupied PH that was characterized by an absence of submerged macrophytes, no predator (*C. argus*) and lower interspecific competition (i.e., the presence of other small-bodied species including *P. parva*, *H. swinhonis* and *H. leucisculus*). Several sympatric goby species have been found to show spatial segregation in use of microhabitats that differ in water flows (Sone *et al.*, 2006), water depths (Horinouchi, 2008), occurrences of aquatic vegetation (Hernaman & Probert, 2008), types of substrata (Hernaman & Probert, 2008; Horinouchi, 2008), salinities and prey abundances (Gill & Potter, 1993). However, most of these studies were conducted within the native ranges and not much is known about the habitat associations once the species have been introduced into new environments (Keller & Taylor, 2008).

These results in our study are in accordance with the reports from native range lakes along Yangtze River (Xie *et al*., 2000b;Xie *et al*., 2005; Li *et al*., 2010) where *R. giurinus* mainly occupies habitats without vegetation or with simple-structured vegetation (e.g., *Nelumbo nucifera* and *Trapa bispinosa*) located in the central parts of the lakes. Habitat complexity from aquatic plants may be unimportant for benthic small-bodies goby species because they have two pelvic fins that form a small sucking plate and enables attach itself to the lake bottom, and thus are probably less vulnerable to predators compared with those staying off the bottom (Aboul Hosn & Downing, 1994; Xie *et al*., 2000b; Xie *et al*., 2005; Horinouchi, 2007). In Lake Erhai, however, *C. argus* was the only potential predator of the two goby species (Ma & Xie, 1999; Table 1) and its distribution was limited exclusively to LH. Moreover, the other small fish species (e.g. *H. swinhonis*, *P. parva* and *H. leucisculus*, Table 1) also used LH in Lake Erhai, which is similar to what has been observed in native shallow lakes (Xie *et al*., 2005; Li *et al*., 2010). Therefore, *R. giurinus* may have lower predation risk and interspecific competition when using PH in Lake Erhai. In contrast, *R. cliffordpopei* mainly used LH that was characterized as a substantially heterogeneous habitat with abundant preys but stronger predation risks (i.e., *C. argus*) and interspecific competitions with similar fishes.

Habitat segregation is an important strategy that promotes coexistence among sympatric species with niche overlap and it usually lead to multi-dimension resources partitioning involving food, space, shelter or nursery site (Cooper *et al*., 2008; Gross *et al*., 2009). For instance, in Biggijavri lake of Norway, grayling *Thymallus thymallus* were exclusively caught in shallow near-shore areas, feeding chiefly on surface insects and Trichoptera larvae, whereas arctic charr *Salvelinus alpinus* were mainly found along the benthic profile, feeding predominantly on insects and snails (Amundsen *et al*., 2010). The observed habitat segregation of the two goby species may alleviate their food overlap and enhance the coexistence with high abundances in Lake Erhai (Xie *et al*., 2000b; Xie *et al*., 2005; Zhang, 2005). Moreover, species of the genus *Rhinogobius* were male-guard nest spawning and nest site availability is crucial for reproduction (Takahashi & Yanagisawa, 1999; Tamada, 2005). The different habitat preference is also likely to mitigate the competition for nest sites between the two species.

Two possible mechanisms might have led to the observed habitat segregation. First, competition between the two goby species might have driven the exclusion of *R. cliffordpopei* from PH. Strong interspecific competition is likely to occur since the species were found to display a high level of diet overlap in native range lakes along the middle and lower reaches of the Yangtze River (Xie *et al*., 2000a; Xie *et al*., 2005; Zhang, 2005). Indeed, sympatric goby species of the genus *Rhinogobius* have often been observed to show interference competition for resources (Sone *et al*., 2001; Sone *et al*., 2006). For instance, in tributaries of the Shimanto River of Japan, habitat partitioning between *Rhinogobius* sp. LD (large-dark type) and *Rinogobius* sp. CB (cross-band type) is a results of interspecific competition (Sone *et al*., 2001; Sone *et al*., 2006). Alternatively, the two goby species might display differences in habitat use that could lead to selective habitat segregation (Gill & Potter, 1993; Hernaman & Probert, 2008; Horinouchi, 2008), as observed for *Acentrogobius* sp. 1 and *A*. sp. 2 in Lake Hamana, Japan (Horinouchi, 2008).

4.2 Niche separation along trophic axis

The food resources exploitation of the two gobies is substantially affected by life stages and habitats. Thus, the trophic partitioning (i.e. niche separation along food axis) patterns are different among the different ontogenetic stages and habitats. Both gut content analyses (GCA) and stable isotope analyses (SIA) demonstrated that macrozooplankton (Cladocera and Copepoda) were the main preys for juveniles and also important for sub-adults of these two goby species in Lake Erhai. Our findings were consistent with those observed in native lakes along the middle and lower reaches of the Yangtze River including Liangzi Lake (Xie *et al*., 2005) and Biandantang Lake (Xie *et al*., 2000a; Zhang, 2005). When they grow into sub-adults and adults, their trophic niche showed a strong ontogenetic shift as individuals gradually shifted to aquatic insects and Tubificidae. As substrata-associated species, most gobies mainly consume macrozoobenthos when they settle to bottom after pelagic stages (Sone *et al*., 2001; Kakareko *et al*., 2005; Borza *et al*., 2009). Among those macrozoobenthos, Chironomidae larvae are one of the most important preys for species of the genus *Rhinogobius* (Xie *et al*., 2000a; Sone *et al*., 2001; Xie *et al.* 2005; Zhang, 2005; Rusuwa *et al*., 2009). Some other goby species have also been shown a clear ontogenetic diet shift (Kanou *et al*., 2005; Rusuwa *et al*., 2009). In the Ado River of Japan, for instance, the three most important food items of *Rhinogobius brunneus* ‘orange’ for juveniles are Ephemeroptera, Diptera and Cyanophyta but became Ephemeroptera, Trichoptera and Detritus for adults(Rusuwa *et al*., 2009).

For food partitioning, small-sized juveniles of the two gobies in littoral habitat (LH) showed similar trophic niche but medium-sized sub-adults and large-sized adults displayed different trophic niches. The body size of fishes has a strong trophic effect since it is a pivotal determinant affecting individual’s dispersal abilities, mortality risks (Einum *et al*., 2006), foraging efficiencies and/or competitive abilities (Hjelm & Persson, 2001; Colloca *et al*., 2010; Nakayama & Fuiman, 2010). Small individuals at early ontogeny are small-sized, gape-limited and thus are almost exclusively pelagic and zooplanktivorous. Although they may strongly compete for a limited food resource during this period, such a competition rarely results in trophic niche specialization (Nakayama & Fuiman, 2010; Colloca *et al*., 2010). In later ontogenetic life stages, however, fishes show much broader prey size spectrums. Moreover, large-sized individuals may have better locomotor capacity and higher feeding efficiency (Hjelm & Persson, 2001; Borcherding *et al*., 2010). Therefore, they are able to exploit different preys and thus can specialize and partition food resources, which may allows coexistence rather than a competitive exclusive among closely-related or ecological similar species within a community (Colloca *et al*., 2010; Davis *et al*., 2012). Several studies have demonstrated that goby species (sub-adults or adults) exhibited food resources partitioning when they become abundant out of native ranges (Kakareko *et al*., 2005; Borza *et al*., 2009; Borcherding *et al*., 2012). In the River Danube of Hungary, for instance, bighead goby mainly consumed *Dikerogammarus* spp but round goby preferred Chironomidae larvae (Borza *et al*., 2009).

In profundal habitat (PH), SIA indicated that sub-adults and adults showed similar trophic niche between species, whereas their diet composition was different using GCA. Since GCA provides a snapshot of diets reflecting an individual’s recent and opportunistic diets but SIA is an integrative tool to elucidate long-term dietary records (Post, 2002; Polito *et al*., 2011; Cucherousset *et al*., 2012), the results of SIA were probably more reliable. Thus, sub-adults and adults of the two species displayed different patterns of food resources partitioning between LH and PH. Dietary resource exploitations among competing fishes may vary with environmental factors including habitat conditions (e.g. stable *vs* highly variable ecosystem, Davis *et al*., 2012), prey availabilities (Shimose *et al*., 2010), density of competitors (Kaspersson *et al*., 2010), shape of food patches and group size of competitors (Kim & Grant, 2007). In Lake Erhai, LH and PH show a large difference in habitat conditions (e.g. water depths and densities of submerged macrophytes), food abundances, densities of competitors and predators, etc. Therefore, further investigations are needed to elucidate the specific effects of those factors on patterns of competition and/or food partitioning between the two species.

4.3 Niche separation along temporal axis

The two gobies showed niche separation along temporal axis in terms of reproduction cycles, which resulted in the different time in appearance of similar-sized cohorts and different populations dynamics. However, no temporal partitioning was observed in diel and seasonal activity and feeding rhythm between them.

R. cliffordpopei and *R. giurinus* differed in their reproductive biology, notably in the spawning cycles with differences in the onset of spawning and the peak of spawning seasons in Lake Erhai. The spawning cycles of the two species are highly variable in their native range along the middle and lower reaches of the Yangtze River. In Lake Biandangtang, for instance, spawning seasons of both species occur from the middle of April to late July (Zhang, 2005), whereas *R. giurinus* spawns from July to September in Lake Chaohu (coexisting with *R. cliffordpopei*) and Lake Dongting (without *R. cliffordpopei*, Yan & Chen, 2007). When introduced into Lake Fuxian, *R. giurinus* spawned from July to October (Yan & Chen, 2007). Because of the different reproduction cycles, the cohort structures of the two species revealed that the newly born individuals appeared at different months (i.e. new born cohort appeared in April for *R. cliffordpopei* and in July for *R. giurinus*), which directly resulted in the different body sized cohorts at the same time between the two gobies. These differences can mitigate the food competition of the similar sized individuals between species since they consumed different foods at different life stages. Moreover, this partitioning is particularly important for individuals at early ontogeny because they are small-sized, gape-limited and thus are almost exclusively zooplanktivorous. They strongly compete for a limited food resource during this period (Nakayama & Fuiman, 2010; Colloca *et al.*, 2010). In addition, our findings indicated that seasonal changes of population abundances were different between the two species. CPUE of benthic fyke nets showed significantly lower values in April to June for *R. cliffordpopei* and in July to September for *R. giurinus*. This pattern strongly coincided with the death of adults after spawning. After these periods, the CPUE increased clearly resulting from the newly settled juveniles. The highly abundant populations of the two species occurred at different periods in a one-year period, which is also a mechanism promoting temporal partitioning between the two species.

In SH, our results demonstrated that there was no temporal and food partitioning. Generally, niche differentiation along spatial dimension is more important than trophic dimension that is more common than temporal dimension (Schoener, 1974). In Lake Erhai, habitat segregation is probably the main mechanism for the coexistence of the two competing gobies. Several gobies are evidenced to partition food resources when they become abundant within an ecosystem (Kakareko *et al.*, 2005; Borza *et al.*, 2009; Borcherding *et al.*, 2012). In Lake Erhai, the two gobies display food partitioning in LH where the ecological similar species (e.g. *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873)) are highly abundant. The densities of the two gobies and other ecological similar species are low in SH and PH, where the food resources may be sufficient for them. For temporal partitioning, Kronfeld-Schor & Dayan (2003) argued that it would be a viable mechanism for reducing resource competition when the shared limiting resources differ between activity times (particularly for those species whose prey populations show activity patterns) or when the limiting resources are renewed within the time involved in the separation. In this study, macrozooplankton may display diel vertical migration that can cause changes of food resources at bottom among different activity times (Hay, 2003; Williamson *et al.*, 2011). However, aquatic insects do not show diel variations and these two main food items seems to not show diel renewed patterns. Therefore, further laboratory investigations are needed to provide a better understanding of their feeding behaviors and competitive interactions under various food resource levels (e.g. limited *vs* saturated) and/or different changing patterns of food resources (e.g. stimulating diel vertical migration of macrozooplankton or seasonal variation of aquatic insects).

4.4 Implications for management

We found the abundance of *R. giurinus* were very high in PH, relatively low in SH and particularly low in LH, whereas abundance of *R. cliffordpopei* were the highest in LH and particularly low in PH and SH (Fig. 2). Therefore, the management programs should be mainly in PH and SH for controlling *R. giurinus* and in LH for controlling *R. cliffordpopei.*

In Lake Erhai, the two gobies showed obvious differences in spawning cycles and also a highly variable spawning compared with the other populations in the native and introduced ranges. Therefore, regionally specific investigations of these two species spawning cycles will be critical before any control operations are put into effect. Our study was the first to demonstrate that the two species showed different spawning cycles within the same ecosystem outside of their native range, and this is a crucial issue for the timing of deployment of remediation actions (Ling, 2002; Britton *et al.*, 2011). Management operations for fishes are often more effective when implemented pre-spawning because offsprings usually increase several orders of magnitude population size and disperse intensively within the ecosystem (Ludgate & Closs, 2003; Wimbush *et al.*, 2009). Moreover, the removal of adults soon after spawning may result in a population increase in juveniles (e.g. decreased intraspecific competition) rather than their decline. In New Zealand, for example, Ludgate & Closs (2003) revealed that the numbers of young-of-the-year Eurasian perch (*Perca fluviatilis*) in ponds increased markedly after the cannibalistic adult perch were removed after spawning. In the present study, control measures (e.g. eradication actions or physical removals) are probably most effective from September to February for *R. cliffordpopei*, and from January to April for *R. giurinus*.

Moreover, cohort structures and growth pattern revealed that the newly born individuals of the two species appeared at different months by benthic fyke nets (i.e. new born cohort appeared in April for *R. cliffordpopei* and in July for *R. giurinus*). Those differences are important for determining the mesh size of benthic fyke nets in physical removals, timing of deployment of remediation actions as well as controlling methods in different seasons (Knapp & Matthews, 1998; Ling, 2002; Britton *et al.*, 2011). Specifically, in order to remove the small-sized individuals, the mesh sizes of benthic fyke nets should be less than 4 mm from March to June for *R. cliffordpopei* and from June to August for *R. giurinus* because our empirical experiences indicated that nets with mesh sizes of 4 mm were inefficient for those individuals. In addition, most small-sized individuals (i.e. fish larvae) are pelagic from February to June for *R. cliffordpopei* and from April to August for *R. giurinus*, the benthic fyke nets are useless before these fish larvae growing big enough to settle into bottom. In these periods, other controlling methods are very likely to be more efficient than benthic fyke nets, such as chemical treatments (e.g. rotenone, Ling, 2000), electricity fishing (Britton *et al*., 2011), and/or light trapping (Meekan *et al*., 2001; Vilizzi *et al*., 2008).

The fluctuations of mean body length of the two species were apparently different (*R. cliffordpopei* showed the highest values in March and lowest June or July, while *R. giurinus* showed the lowest values in June and lowest in August). Combined the patterns of cohort structures, monthly changes of mean body length is the most important criterion for mesh size of benthic fyke nets used for physical removals.

The information on monthly changes of CPUE in this study should be incorporated into the management strategies from the perspective of cost-efficiency since the main controlling actions in the lake are currently the physical removals by fyke nets. For *R. cliffordpopei*, the physical removals in winter are recommended to be redoubled despite the significantly lower CPUE because the actions during this period aim at decreasing the mature populations as stated above. Moreover, because *R. cliffordpopei* mostly uses the littoral zones of the lake, the low abundance of submerged macrophytes in winter can facilitates an easy setting and retrieving of nets. For *R. giurinus*, however, physical removals are suggested to be lessened or concealed during winter as such operations in profundal zones (water depths range 12 – 20 m) are energy-costly and time-costly. In the other low CPUE periods (i.e. from April to June for *R. cliffordpopei* and from July to September for *R. giurinus*), physical removals using fyke nets probably can be transferred to other outlets (e.g. electricity fishing, light trap, etc.). In contrary, removal actions should be strengthened when the CPUE are high July to September for *R. cliffordpopei* and from April to June, September to November in *R. giurinus.*

4.5 Conclusion and perspectives

The present study represents an empirical case study demonstrating that two congeneric and invasive gobies species show niche separation along spatial, trophic and temporal axes, which is a major mechanism facilitating a stable coexistence of the two species at high abundances within an ecosystem. The niche separation along habitat is important one in the lake for decreasing resources competition. The second is the niche separation along time (temporal partitioning) in terms of the different reproduction cycles and thus the different population dynamics. Since these two species showed a highly plastic and opportunistic feeding strategy and a wide spectrum of prey, the niche separation along trophic axis seems to be less important.

However, further investigations are suggested to examine how exploitive competition (e.g. food intake, feeding rate) or interference competition behaviors (e.g. chase rate, attack rate, food holding capacity) varies with habitat conditions such as food resource levels (e.g. limited *vs* saturated), population densities, predation risks, etc. Those investigations should provide a full understanding of competitive interactions between the two species.

Furthermore, since the current control operations taken in Lake Erhai are almost only physical removals by fyke nets, the synergistic remediation are highly recommend as a complementary strategy such as chemical treatments, electricity fishing removals, light trapping and/or bio-controlling (Ling, 2000; Britton *et al.*, 2011). Studies are needed to quantify the efficiency of these synergistic programs and evaluate their potential effects on non-target species and introduced ecosystem, especially for chemical treatments and bio-controlling.

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Part II: Publications

Habitat segregation between two congeneric and introduced goby species

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Article

Habitat segregation between two congeneric and introduced goby species

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With 3 figures and 6 tables

Abstract: Spatial segregation is one of the most important mechanisms that facilitates coexistence among competing species. Large populations of two introduced and congeneric goby species (*Rhinogobius giurinus* and *Rhinogobius cliffordpopei*) now co-occur in Lake Erhai, a plateau lake in the Yunnan-Guizhou Plateau (China). Herein we quantified the spatio-temporal distribution of the two species to determine whether spatial segregation occurred within the same ecosystem. A total of 67,819 individuals of *R. giurinus* and 36,043 of *R. cliffordpopei* were sampled across four seasons. The results indicated that *R. giurinus* mostly occupied profundal habitat (PH) while *R. cliffordpopei* mainly used littoral habitat (LH). Correlation analysis revealed the abundance of *R. giurinus* was positively associated with deep water, silt and coarse sand substrata, whereas the distribution of *R. cliffordpopei* was positively associated with high densities of macrozooplanktons and high abundances of other fish species, high concentration of dissolved oxygen and high densities of submerged macrophytes. Except in spring, the body condition of *R. giurinus* was significantly higher in the PH than in the LH. The body condition of *R. cliffordpopei* did not differ significantly between habitats in the four seasons. These findings demonstrate that the two congeneric and introduced goby species occupy distinct habitats, indicating that spatial segregation enables coexistence of the two invasive species at high abundances within an ecosystem.

Key words: habitat segregation, biological invasions, body condition, ecological niche.

Introduction

Phylogenetically-related or ecologically-similar species often show separation along niche axes involving several dimensions such as habitat, food and time (Friberg et al. 2008, Gross et al. 2009, Amundsen et al. 2010). Habitat is arguably the most important dimension that can promote resource partitioning and has often been implicated as a primary mechanism for coexistence among sympatric species with similar ecological functions (Hernaman & Probert 2008, Hesselschwerdt et al. 2008, Amundsen et al. 2010). Habitat segregation has been widely observed in fishes including closely related species (Sone et al. 2006, Horinouchi 2008, Hernaman & Probert 2008), different age and size classes (Johnsona et al. 2011), or polymorphic populations (Kahilainen et al. 2004). As substrata-associated species, most goby species show

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limited capacity for quick and/or long distance swimming after they settle in the benthos and habitat use has substantial effects on access to trophic resources and individual performances (Hayden & Miner 2009). Several sympatric goby species have been found to show spatial segregation in use of microhabitats that differ in water flows (Sone et al. 2006), water depths (Horinouchi 2008), aquatic vegetation (Hernaman & Probert 2008), types of substrata (Hernaman & Probert 2008, Horinouchi 2008), salinities and prey abundances (Gill & Potter 1993). However, most of these studies were conducted within the native ranges and not much is known about the habitat segregation once the species have been introduced into new environments (Keller & Taylor 2008).

Rhinogobius giurinus (Rutter, 1897) and *Rhinogobius cliffordpopei* (Nichols, 1925) are small-bodied (maximal total body length < 80 mm) and ecologically similar species with comparable life histories and feeding habits in native lakes along the middle and lower reaches of the Yangtze River in China (Zhang 2005, Wu & Zhong 2008). They usually spawn from April to June with a life span of one-year in native lakes (Xie et al. 2005, Zhang 2005). Their diet is mainly composed of large zooplankton (Cladocera and Copepoda) and aquatic insects (Chironomidae larvae) (Xie et al. 2000b, Xie et al. 2005, Zhang 2005). *Rhinogobius giurinus* is associated with vegetation-free or simple-structured vegetation habitats in native lakes (Xie et al. 2000a, Xie et al. 2005, Li et al. 2010). In those lakes, however, *R. cliffordpopei* has been reported to occur only at very low densities and no information is available about habitat selection when the two species co-occur in their native areas. Both species were introduced inadvertently into Lake Erhai in 1961, populations densities rapidly increased in the 1970s and the two gobies are now the most dominant fish species in the lake (Du & Li 2001). Therefore, they provide an excellent opportunity to study habitat segregation of congeneric species at very high abundances.

The aim of the present study was to determine if spatial segregation occurs when two introduced goby species coexist in high abundances. We also examined how habitat occupancy affects the individual performance of each species. Specifically, the following questions were examined: (1) does the abundances of the two goby species differ across the habitats and seasons; (2) how are the abundances of the two goby species associated with environmental characteristics and (3) does habitat use influence individual performance (i.e., body-condition) differently for the two species?

Material and methods

Study sites and habitat characteristics

Lake Erhai (105° 5–17′ E, 23° 35–58′ N) is a freshwater plateau lake with a surface area of 250 km^2 and a catchment area of 2,600 km^2 in Yunnan-Guizhou Plateau of China (Fig. 1). The maximum water depth is approximately 21 m without thermal or dissolved oxygen stratification. Twenty-eight fish species have been found in the lake in recent years and most of them are planktivorous. The dominant species are small-bodied fishes, especially non-native species, including freshwater gobies *R. giurinus* and *R. cliffordpopei*, *Neosalanx taihuensis* (Chen, 1956), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873) and *Hemiculter leucisculus* (Basilewsky, 1855). *Channa argus* (Cantor, 1842) is the only recorded piscivorous fish species. *R. giurinus* and *R. cliffordpopei* are the most abundant benthic fish species with annual yields representing about 48 % of total fish yields (kg) in the lake.

Habitat characteristics of the lake were investigated prior to fish sampling (Table 1). For each sampling site, water temperature, dissolved oxygen and conductivity were measured *in situ* using a handheld meter (YSI Model Pro20, Ohio, USA). A handheld pH meter was used to measure pH *in situ* (YSI Model EcoSense pH10 A, Ohio, USA). Total nitrogen, total phosphorus and Chlorophyll-*a* were determined using a standard colorimetric method (APHA et al. 1995). Macrozooplanktons were collected by hand-nets (mesh size 64 μm) and counted under a dissecting microscope. Substrata structures were sampled by Peterson dredge (0.0625m^2) and determined by macroscopic appearance. Biomass (wet weight) of submerged macrophytes was determined using a 30 cm × 50 cm clamp at three random measurements within each sampling site. Habitats for benthic fishes in Lake Erhai were classified into three types: 1) littoral habitat (LH, water depth less than 6 m, high abundance of submerged macrophytes, submersed-macrophyte detritus substrata); 2) sub-littoral habitat (SH, water depths range 6 to 12 m, few submerged macrophytes, submersed-macrophyte detritus and silt substrata); and 3) profundal habitat (PH, water depths ranges 12 to 20 m, no submerged macrophytes, silt and coarse sand substrata).

Fish sampling and data collection

Five (LH1-LH5), four (SH1-SH4) and three (PH1-PH3) sites were sampled for each habitat in the middle and northern section of the lake (Fig. 1, Table 1). Fish sampling was carried out in the first week of February (winter), May (spring), August (summer) and November (autumn) of 2010 using benthic fyke nets. The net comprised a trunk stem with twenty traps, two end traps and two end pockets. Total length of the net was 15 m, including 12 m of trap (0.6 m for each trap), 2 m of end trap (1 m for each trap) and 1 m of end pocket (0.5 m for each one). The framework of each trap was made of iron wire with the width of 0.35 m and the height of 0.62 m. The end trap was round and the diameter gradually decreased from 0.3 m to 0.1 m. The mesh size of all nets was 4 mm. At each sampling site, eight nets were deployed separately with a stone in each end of the nets. After 24 hours, the catches in the end pockets were collected and rapidly transferred to −20 °C in the laboratory. In each season, the nets were deployed at 8:00 to 10:00 am and we sampled three sites per day (twenty four nets were used each day). The order

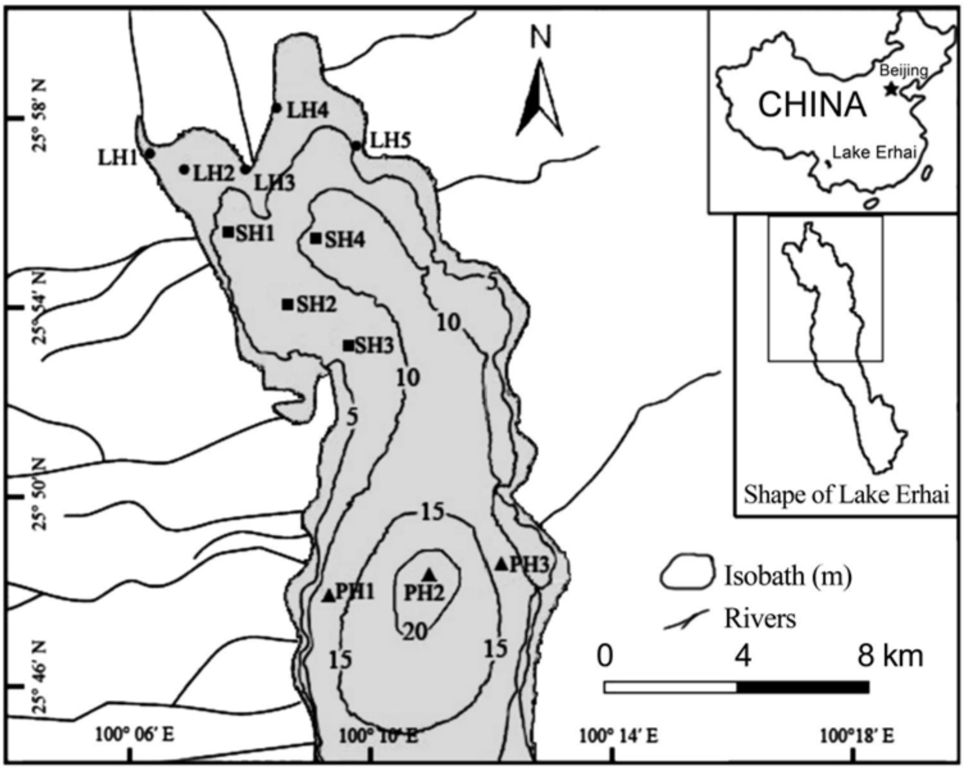

Fig. 1. Sample sites in the three habitats located in the central and northern section of Lake Erhai (China). Five (LH1-LH5), four (SH1-SH4) and three (PH1-PH3) sites were sampled in littoral (●), sub-littoral (■) and profundal (▲) habitats, respectively.

of site sampling was identical in the four seasons. Hence, fish sampling lasted four days in each season. All the procedures complied with Chinese legislation.

All fishes collected in each fyke net were identified to species level, counted and batch-weighed. A random sub-sample from homogeneous catches of the total catches at each habitat in each season was preserved in 8 % formalin for two weeks and then transferred to 75 % ethanol for storage. About 1000 individuals of *R. giurinus* and 400 individuals of *R. cliffordpopei* (only around 400 individuals of *R. cliffordpopei* were sampled in PH in winter and autumn) were measured for each habitat and each season. Total length (TL, nearest mm) and body mass (BM, nearest 0.01 g) of individuals from the sub-samples were measured after preservation. Since preservation can affect the length/mass ratio, all subsequent analyses were performed based on preservation-corrected TL and BM. Specifically, TL and BM were measured individually before and after preservation for 100 specimens and the relationships between fresh and preserved TL and BM were established ($TL_{Fresh} = 1.007\ TL_{Preserved} + 0.417$, $R^2 = 0.956$ and $BM_{Fresh} = 0.877\ BM_{Preserved} + 0.011$, $R^2 = 0.967$, $n = 100$). These equations were then used to calculate preservation-corrected TL and BM.

Statistical analysis

Goby abundances in the three habitats were estimated using catch per unit effort (CPUE), defined as the number of individuals caught in one net per day (ind. net^{-1} day^{-1}). Differences in goby abundances (i.e., CPUE) between habitats and seasons were tested using repeated-measures ANOVA (RM-ANOVA) with habitats as a fixed factor and seasons as a random factor. Sphericity assumption was tested using Mauchly's test and the degrees of freedom were adjusted by Greenhouse–Geisser Epsilon when data violated the assumption of sphericity. Because the interactions between habitats and seasons were significant (Table 2), one-way ANOVAs with Tukey's multiple comparisons were subsequently used to test the differences in goby abundances between habitats in each season separately. Data of goby abundances were log-transformed to achieve normality and homoscedasticity.

Correlation analyses with Pearson correlation coefficients were used to explore how goby abundances were associated with environmental variables. Correlation analyses were conducted separately for the two goby species. Environmental variables and goby abundances were averaged in each season in correlation analysis.

Residuals from a linear regression of TL (log-transformed) and BM (log-transformed) were used as an index of body condition (Schulte-Hostedde et al. 2005). A positive body condition value indicated that the individual had a heavier body mass related to body length compared to the rest of the population. Differences in body condition of the two goby species between habitats and seasons were tested using General Linear Model (GLM) procedures with habitats as a fixed factor and seasons as a random factor. GLM were run initially with the interaction term (full model) between habitats and seasons and again with-

Table 1. Environmental variables measured in the three habitats in Lake Erhai (China). Reported values are mean ± standard deviation.

Environmental variables	Littoral habitat (LH)	Sub-littoral habitat (SH)	Profundal habitat (PH)
Water depth (m)	3.11 ± 1.17 (n = 40)	8.13 ± 1.35 (n = 32)	15.92 ± 3.14 (n = 24)
Water temperature (°C)	16.92 ± 4.74 (n = 40)	17.14 ± 4.48 (n = 32)	16.91 ± 4.64 (n = 24)
Submerged macrophytes (g m^{-2})	3307.35 ± 881.32 (n = 40)	102.66 ± 49.49 (n = 32)	0 (n = 24)
pH	9.33 ± 0.30 (n = 40)	9.20 ± 0.33 (n = 32)	9.08 ± 0.26 (n = 24)
Secchi depth (cm)	214.17 ± 35.91 (n = 40)	235.43 ± 22.47 (n = 32)	254.66 ± 25.43 (n = 24)
Conductivity (µS cm^{-1})	237.33 ± 167.50 (n = 40)	232.62 ± 11.89 (n = 32)	233.87 ± 12.76 (n = 24)
Dissolved oxygen (mg L^{-1})	9.23 ± 1.05 (n = 40)	7.56 ± 1.08 (n = 32)	7.51 ± 0.88 (n = 24)
Total nitrogen (mg L^{-1})	0.46 ± 0.12 (n = 20)	0.42 ± 0.08 (n = 16)	0.43 ± 0.05 (n = 12)
Total phosphorus (mg L^{-1})	1.38 E-2 ± 0. 81 E-2 (n = 20)	1.26 E-2 ± 0.77 E-2 (n = 16)	1.42 E-2 ± 0.69 E-2 (n = 12)
Chlorophyll-a (mg L^{-1})	8.27 ± 2.88 (n = 20)	12.12 ± 3.82 (n = 16)	12.44 ± 3.09 (n = 12)
Macrozooplankton (ind. L^{-1})	296.43 ± 60.56 (n = 20)	198.62 ± 35.48 (n = 16)	92.65 ± 27.43 (n = 12)
Substrata structures [1]	–1	0	1
CF [2]	32.91 ± 8.16 (n = 160)	7.67 ± 2.74 (n = 128)	0.16 ± 0.09 (n = 96)
Pseudorasbora parva [3]	8.83 ± 4.55 (n = 160)	1.52 ± 0.65 (n = 128)	0 (n = 96)
Hypseleotris swinhonis [3]	12.51 ± 5.58 (n = 160)	2.08 ± 1.41 (n = 128)	0 (n = 96)
Hemiculter leucisculus [3]	5.41 ± 2.10 (n = 160)	1.87 ± 0.94 (n = 128)	0.16 ± 0.09 (n = 96)
Channa argus [4]	1.46 ± 1.12 (n = 160)	0.34 ± 0.22 (128)	0 (96)

[1] Type of submersed macrophyte detritus, mixture of submersed macrophyte detritus and silt, mixture of silt and coarse sand were assigned value of –1, 0 and 1, respectively.

[2] Total abundances (ind. net^{-1} day^{-1}) of all fish species except *R. giurinus* and *R. cliffordpopei* caught in fyke nets.

[3] Abundances (ind. net^{-1} day^{-1}) of the other dominant species (species with relative abundance ≥ 10 % of total catch) caught in fyke nets

[4] Abundances (ind. net^{-1} day^{-1}) of piscivorous fish species (i.e., *Channa argus*) in fyke nets.

out the interaction (simplified model) if it was not significant. Because the interactions between habitats and seasons was significant for *R. giurinus*, one-way ANOVAs with Tukey's multiple comparisons were subsequently used to test the differences in body condition between habitats in each season separately. Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, USA) and the significance was determined at $p < 0.05$ in all cases.

Results

Goby abundances across habitats and seasons

A total of 67,819 individuals of *R. giurinus* and 36,043 individuals of *R. cliffordpopei* were sampled across four seasons. The abundances of the two goby species were significantly influenced by habitat, season,

Table 2. Repeated-measures ANOVAs (RM-ANOVA) used to test differences in the abundances of *R. giurinus* and *R. cliffordpopei* between habitats (fixed factor) and seasons (random factor) in Lake Erhai (China).

Species	Sources	d. f.	F	*p*
R. giurinus	Habitats	2	288.436	<0.001
	Error	93		
	Seasons	2	46.508	<0.001
	Error	172		
	Habitats × Seasons	4	19.984	<0.001
R. cliffordpopei	Habitats	2	221.213	<0.001
	Error	93		
	Seasons	2	122.403	<0.001
	Error	213		
	Habitats × Seasons	5	36.529	<0.001

and the interaction between habitat and season (RM-ANOVA, Table 2). The abundance of *R. giurinus* significantly differed between habitats across the four seasons (one-way ANOVAs, Table 3). Specifically, the abundance of *R. giurinus* was significantly higher in PH than in LH and SH for all seasons (Tukey HDS, $p<0.001$) and in SH than in LH (Tukey HDS, $p<0.001$) except in winter (Tukey HDS, $p=0.079$) (Fig. 2). The abundance of *R. cliffordpopei* also significantly differed between habitats across the four seasons (one-way ANOVAs, Table 3). In spring, autumn and winter, the abundance of *R. cliffordpopei* was significantly higher in PH than in SH and in LH (Tukey HDS, $p<0.001$), whereas there were no significant differences between LH and SH (Tukey HDS, $p=0.132$, 0.095 and 0.422, respectively). In summer, the abundance of *R. cliffordpopei* was significantly different between the three habitats (Tukey HDS, LH vs SH, $p=0.016$, LH vs PH, $p<0.001$, SH vs PH, $p<0.001$) (Fig. 2).

Goby abundances in relation to environmental variables

A strong association between environmental variables and goby abundances was observed for the two species. Specifically, Pearson correlation coefficients (r) demonstrated that the abundances of *R. giurinus* were positively associated with water depth ($r=0.667$, $p<0.001$), silt and coarse sand substrata ($r=0.640$, $p<0.001$). In contrast, the abundances of *R. cliffordpopei* were positively correlated with submerged macrophytes ($r=0.662$, $p<0.001$), abundances of other fish ($r=0.606$, $p<0.001$), densities of macrozooplankton ($r=0.363$, $p<0.001$), concentrations of dissolved oxygen ($r=0.323$, $p<0.001$), submersed macrophyte detritus substrata ($r=-0.608$, $p<0.001$) (Table 4).

Individual performances and habitat use

Habitats, seasons and their interaction term had significant effects on the body condition of *R. giurinus* (RM-ANOVA, Table 5). Body condition of *R. giurinus* differed significantly in summer, autumn and winter but not in spring (one-way ANOVAs, Table 6). In summer and autumn, *R. giurinus* had significantly higher body condition in PH compared with LH (Tukey HDS, $p=0.001$ and 0.001, respectively) and SH (Tukey HDS, $p<0.001$ and $p=0.005$, respectively) (Fig. 3, Table 6). In winter, body condition of *R. giurinus* was significantly lower in LH

Table 3. One-way ANOVAs used to test the differences in the abundances of *R. giurinus* and *R. cliffordpopei* between habitats for each season in Lake Erhai (China).

		R. giurinus			*R. cliffordpopei*		
Seasons	Sources	d.f.	F	*p*	d.f.	F	*p*
Spring	Between Habitats	2	82.014	<0.001	2	139.383	<0.001
	Within Habitats	93			93		
	Total	95			95		
Summer	Between Habitats	2	239.734	<0.001	2	179.449	<0.001
	Within Habitats	93			93		
	Total	95			95		
Autumn	Between Habitats	2	216.930	<0.001	2	131.972	<0.001
	Within Habitats	93			93		
	Total	95			95		
Winter	Between Habitats	2	65.013	<0.001	2	5.286	0.007
	Within Habitats	93			93		
	Total	95			95		

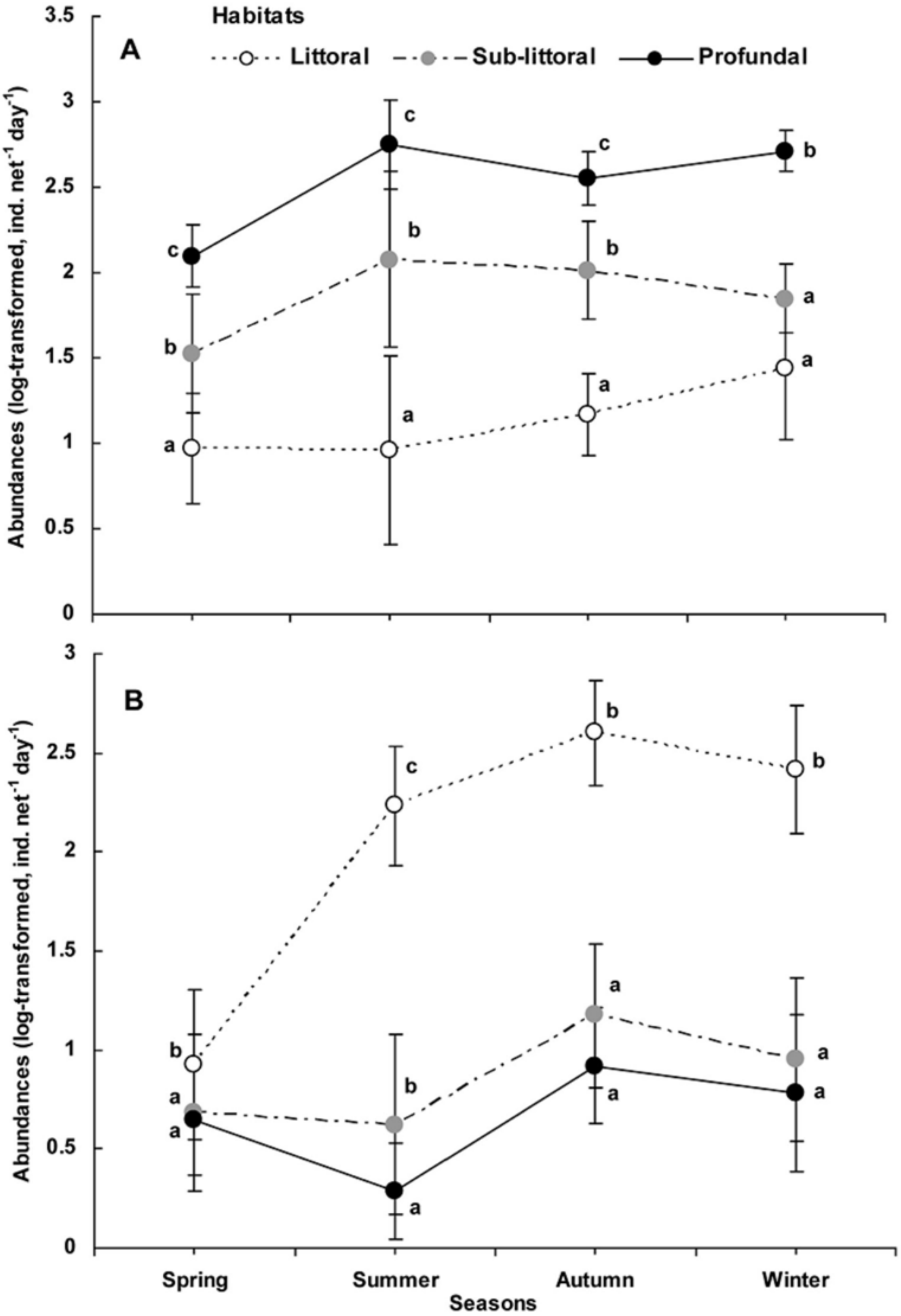

Fig. 2. Mean abundances (log-transformed) of *R. giurinus* **(A)** and *R. cliffordpopei* **(B)** across habitats and seasons (sampling sizes were 40, 32 and 24 for littoral, sub-littoral and profundal habitat in each season, respectively) in Lake Erhai (China). The abundances in the same season with different letters were significantly different. Error-bars represent the standard deviations.

compared to SH (Tukey HDS, $p < 0.001$) and PH (Tukey HDS, $p = 0.027$, Table 6). The body condition of *R. cliffordpopei* differed significantly between seasons ($p < 0.001$) but not between habitats ($p = 0.095$, RM-ANOVA, Table 5) (Fig. 3).

Discussion

The present study demonstrated that two congeneric and introduced freshwater goby species display a strong level of habitat segregation in Lake Erhai.

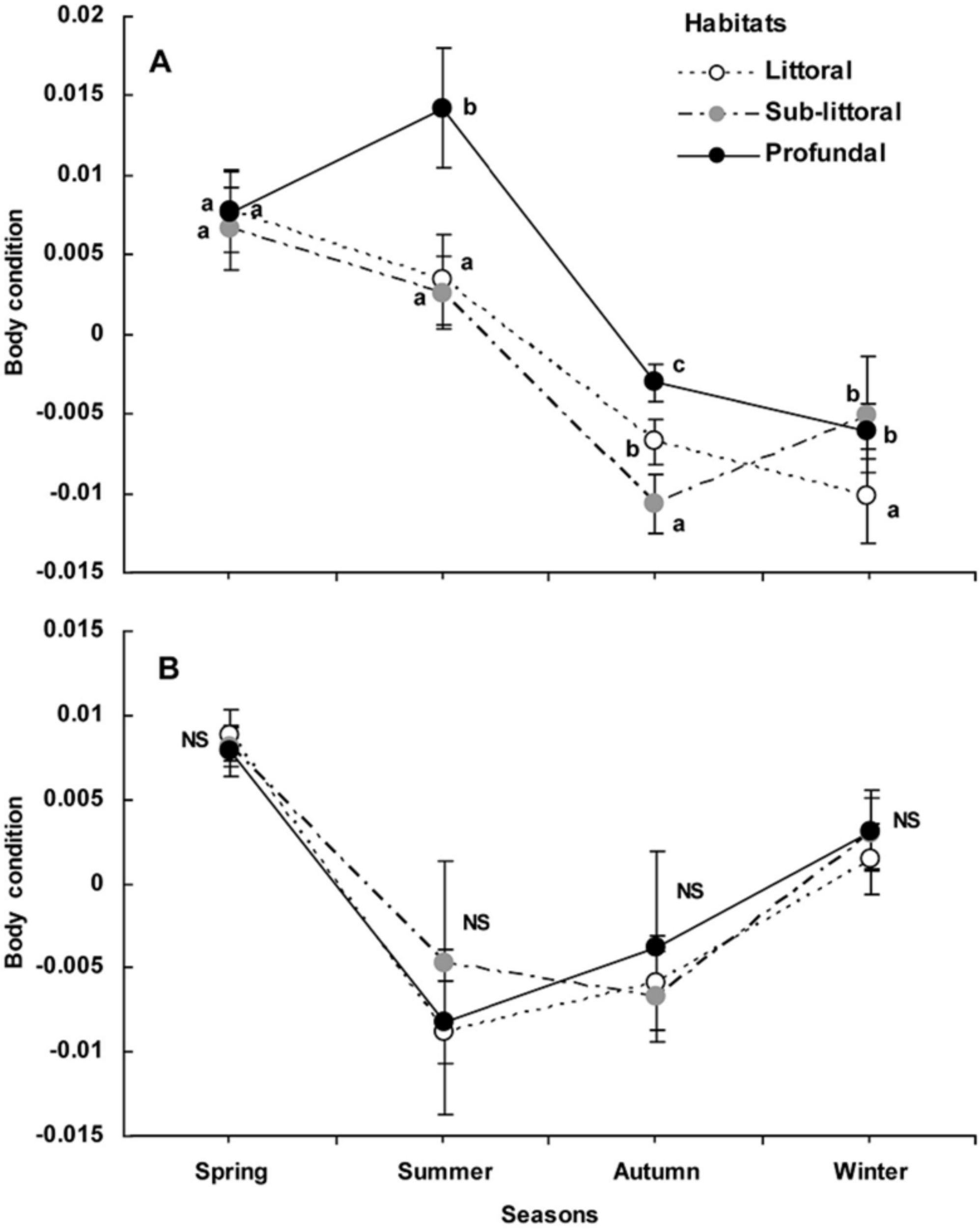

Fig. 3. Body condition of *R. giurinus* (**A**, n = 1000 individuals in each habitat and season) and *R. cliffordpopei* (**B**, n = 400 in each habitat and season) across habitats and seasons in Lake Erhai (China). Body conditions in the same season with different letters were significantly different (NS: not significantly different). Error-bars represent the standard deviations.

R. giurinus mostly occupied PH that was characterized by an absence of submerged macrophytes, no predator (*C. argus*) and lower interspecific competition (i.e., the presence of other small-bodied species including *P. parva*, *H. swinhonis* and *H. leucisculus*). These results are in accordance with the reports from native range lakes along the Yangtze River (Xie et al. 2000a, Xie et al. 2005, Li et al. 2010) where *R. giurinus* mainly occupies habitats without vegetation or with simple-structured vegetation (e.g., *Nelumbo nucifera* and *Trapa bispinosa*) located in the central parts of the lakes. Habitat complexity from aquatic plants may be unimportant for benthic small-bodied goby species because they have two pelvic fins that form a small sucking plate enabling attachment to the lake bottom, and thus are probably less vulnerable to predators compared with those staying off the bottom (Aboul Hosn & Downing 1994, Xie et al. 2000a, Xie et al. 2005, Horinouchi 2007). In Lake Erhai, however, *C. argus* was the only potential predator of the two

Table 4. Correlation analyses (Pearson correlation coefficients (*r*) and *P*-values) between the abundances of *R. giurinus*, *R. cliffordpopei* and environmental variables in Lake Erhai (China).

	R. giurinus		*R. cliffordpopei*	
Environmental variables	r	*p*	r	*p*
Water depth (m)	0.677	<0.001	–0.572	<0.001
Water temperature (°C)	0.169	0.001	0.174	0.001
Submerged macrophytes (g m^{-2})	–0.448	<0.001	0.662	<0.001
pH	–0.053	0.300	0.093	0.068
Secchi depth (cm)	0.209	<0.001	–0.224	<0.001
Conductivity (μS cm^{-1})	–0.015	0.743	0.051	0.318
Dissolved oxygen (mg L^{-1})	–0.226	<0.001	0.323	<0.001
Total nitrogen (mg L^{-1})	–0.089	0.064	0.083	0.104
Total phosphorus (mg L^{-1})	0.013	0.794	0.097	0.084
Chlorophyll–a (mg L^{-1})	0.188	<0.001	–0.010	0.894
Macrozooplankton (ind L^{-1})	–0.524	<0.001	0.363	0.011
CF[1]	–0.452	<0.001	0.606	<0.001
Substrata structures[2]	0.640	<0.001	–0.608	<0.001

Table 5. General Linear Model used to test differences in body condition of *R. giurinus* and *R. cliffordpopei* between habitats (fixed factor) and seasons (random factor) in Lake Erhai (China).

Species	Sources	d.f.	F	*p*
R. giurinus Full model	Habitats	2	6.266	0.002
	Error	7		
	Seasons	3	13.644	0.004
	Error	6		
	Habitats × Seasons	6	8.269	<0.001
	Error	11855		
R. cliffordpopei Full model	Habitats	2	0.008	0.992
	Error	31		
	Seasons	3	23.333	<0.001
	Error	10		
	Habitats × Seasons	6	1.345	0.234
	Error	4613		
Simplified model	Habitats	2	2.359	0.095
	Error	4169		
	Seasons	3	11.653	<0.001
	Error	4169		

goby species (Ma & Xie 1999, Table 1) and its distribution was limited exclusively to LH. Moreover, the other small fish species (e.g., *H. swinhonis*, *P. parva* and *H. leucisculus*, Table 1) also used LH in Lake Erhai, which is similar to what has been found in native shallow lakes (Xie et al. 2000a, Xie et al. 2005, Li et al. 2010). Therefore, *R. giurinus* may have lower predation risk and interspecific competition when using PH in Lake Erhai. In contrast, *R. cliffordpopei* mainly used LH that was characterized as a substantially heterogeneous habitat with abundant prey but stronger predation risks (i.e., *C. argus*) and interspecific competition with similar fishes (Xie et al. 2000a, Li et al. 2010, Neaher et al. 2010).

Habitat segregation is an important strategy that promotes coexistence among sympatric species with niche overlap and it usually leads to multi-dimension resource partitioning involving food, space, shelter or nursery site (Cooper et al. 2008, Friberg et al. 2008, Gross et al. 2009). For example, in Biggijavri lake of Norway, grayling *Thymallus thymallus* (Linnaeus, 1758) were exclusively caught in shallow near-shore areas, feeding chiefly on surface insects and Trichoptera larvae, whereas arctic charr *Salvelinus alpinus*

Table 6. One-way ANOVAs used to test differences in body condition of *R. giurinus* between habitats for each season in Lake Erhai (China).

Seasons	Sources	d.f.	F	*p*
Spring	Between Habitats	2	0.352	0.704
	Within Habitats	3075		
	Total	3077		
Summer	Between Habitats	2	22.656	<0.001
	Within Habitats	2847		
	Total	2849		
Autumn	Between Habitats	2	4.044	0.018
	Within Habitats	2703		
	Total	2705		
Winter	Between Habitats	2	7.987	<0.001
	Within Habitats	3226		
	Total	3228		

(Linnaeus, 1758) were mainly found along the benthic profile, feeding predominantly on insects and snails (Amundsen et al. 2010). Several goby species have been shown to display habitat and food partitioning when they become abundant in non-native ranges (Erös et al. 2005, Borza et al. 2009, Borcherding et al. 2012). The observed habitat segregation of the two goby species may alleviate their food overlap and enhance their coexistence at high abundance in Lake Erhai (Xie et al. 2000b, Xie et al. 2005, Zhang 2005). Therefore, it would be very insightful to determine whether this habitat segregation is potentially associated with trophic niche differentiation. In addition, species of the genus *Rhinogobius* were male-guard nest spawning and nest site availability is crucial for reproduction (Takahashi & Yanagisawa 1999, Tamada 2005). The different habitat preference is also likely to mitigate the competition for nest sites between the two species.

Body condition is known to be an integrative assessment of individual performances and often considered to be important for evaluating habitat quality in fishes (Lloret et al. 2005, Johnson 2007). Occupying high quality habitat usually results in high body condition, which can subsequently affect survivorship, competitive ability and reproductive success (Green 2001, Koops et al. 2004, Lloret et al. 2005). We found that the body condition of *R. giurinus* was significantly higher in PH compared to LH in summer, winter and autumn. So PH might represent a more profitable habitat for this species. Xie et al. (2005) observed a similar pattern where *R. giurinus* showed greater body length and condition factor in the pelagic habitat (less covered by macrophytes) compared to the littoral habitat (heavily covered by macrophytes) in Liangzi Lake. Predation pressure in LH may affect the body condition of *R. giurinus* since individuals exposed to a high predation risk usually spend more time avoiding predators and forage less frequently with food of lower quality (Madin et al. 2010), leading to lower energy intakes and lipid reserves (Walsh et al. 2012). Additionally, the observed lower bo t6dy condition of *R. giurinus* in LH might be caused by the lower feeding efficiency resulting from the visual and swimming barriers created by the stems and foliage of macrophytes, as well as stronger competition with ecologically similar species (Tugend & Allen 2004, Xie et al. 2005). In contrast, the body condition of *R. cliffordpopei* did not vary significantly between habitats, suggesting that the quality of the three habitats was similar for this species in Lake Erhai.

Two possible mechanisms might have led to the observed habitat segregation. First, competition between the two goby species might have driven the exclusion of *R. cliffordpopei* from PH. Strong interspecific competition is likely to occur since the species were found to display a high level of diet overlap in native range lakes along the middle and lower reaches of the Yangtze River (Xie et al. 2000b, Xie et al. 2005, Zhang 2005). Indeed, sympatric goby species of the genus *Rhinogobius* have often been observed to show interference competition for resources (Sone et al. 2001, Ito & Yanagisawa 2003, Sone et al. 2006). For instance, in tributaries of the Shimanto River of Japan, habitat partitioning between *Rhinogobius* sp. LD (large-dark type) and *Rinogobius* sp. CB (cross-band type) is a result of interspecific competition (Sone et al. 2001, Sone et al. 2006). Alternatively, the two goby species might display differences in habitat use that could lead to selective habitat segregation (Gill & Potter 1993, Hernaman & Probert 2008, Horinouchi 2008), as observed for *Acentrogobius* sp. 1 and *A.* sp. 2 in Lake Hamana, Japan (Horinouchi 2008). In conclusion, our study represents an empirical case study demonstrating that two congeneric and highly abundant fish species exhibit a strong level of habitat segregation outside of their native range. However, determining the mechanisms triggering the observed segregation will require further investigations.

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Trophic niche differences between two congeneric goby species: evidence for ontogenetic shift and possible food partitioning

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Abstract

Species-specific specialization in food resource use is one of primary mechanisms facilitating the stable coexistence among competing species within communities. The present study examined the trophic niche relationship of two connegric and highly abundant goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) using gut content analysis (GCA) and stable isotope analysis (SIA) in Lake Erhai (a plateau freshwater lake in southwest of China). The results demonstrated that diet compositions and trophic niche relationship of those two gobies were significantly affected by life stages (juveniles, sub-adults and adults) and habitats (littoral habitat (LH) and profundal habitat (PH)). Specifically, they showed a strong ontogenetic trophic niche shift whereby juveniles mainly consumed macrozooplankton (Cladocera and Copepoda) but sub-adults and adults primarily consumed Chironomidae larvae and Tubificidae. The two species showed similar trophic niche for juveniles in both habitats but different ones for sub-adults and adults in LH. Moreover, the diet compositions of juveniles of both species were stable in different habitats, whereas *R. giurinus* markedly varied diet compositions between habitats for sub-adults and adults.

Key words: niche differentiation, goby, diet compositions, ontogenetic diet shifts, isotopic values,

Introduction

Niche theory predicts that species-specific specialization in resource use is a primary mechanism allowing the stable coexistence among competing species within a local community (Jones et al., 2001; Kylafis and Loreau, 2011). Specialization in food resource is crucial as it may substantially enhance species coexistence by reducing interspecific competition (Chesson, 2000; Kleynhans et al., 2011). In fish, for instance, closely-related species within communities have been demonstrated to display strong food resource partitioning and trophic niche specialization (Colloca et al., 2010; Fanelli et al., 2011; Davis et al., 2012). Since the diet and foraging behavior of fishes are often strongly driven by environmental conditions such as prey availability (Shimose et al., 2010), density of competitors (Kaspersson et al., 2010) and predators (Hammerschlag et al., 2010), the patterns of food partitioning among competing fishes might therefore also vary between habitats that are characterized by different environmental conditions (Davis et al., 2012). However, the potential effects of habitat conditions on food resource use and partitioning amongst competing fishes still need to be further investigated.

Most animals show complex life cycles during which there is often a significant shift in ecological niche among ontogeny (Moll and Brown, 2008; Schreiber and Rudolf, 2008) and conspecific individuals can display varying resource exploitations due to morphological, physiological and/or behavioral differences between life stages (Brickle et al., 2003; Specziár and Rezsu, 2009; Nakayama and Fuiman, 2010). The magnitude and direction of resource partitioning among competing species may therefore differ during ontogeny. Consequently, investigations based on a single life stage could provide inaccurate information to elucidate the interspecific trophic relationship (Moll and Brown, 2008; Schreiber and Rudolf, 2008).

Fishes have complex age/size structured populations and the range of body size between conspecific individuals can sometimes span over several orders of magnitude (Specziár and Rezsu, 2009; Davis et al., 2012). During ontogeny, strong niche shifts can occur, with individuals belonging to different trophic guilds during their life cycle. As a result, the patterns of food resource use amongst co-occurring fishes are often made more complex by ontogeny-dependent interactions (Nakayama and Fuiman, 2010; Davis et al., 2012). Consequently, investigating food resource use between competing fish species at different life stages is crucial for establishing a full understanding of their trophic relationship (Brickle et al., 2003; Marshall et al., 2008).

Rhinogobius cliffordpopei (Rutter, 1897) and *Rhinogobius giurinus* (Nichols, 1925) are two ecological-similar species with similar life histories and diets in native lakes along the middle and lower reaches of the Yangtze River (Xie et al., 2000; Xie et al., 2005, Zhang, 2005; Wu & Zhong, 2008). Both species were simultaneously introduced into Lake Erhai in 1961, flourished greatly from the 1970s and have become the most dominant benthic species of the lake (Du and Li, 2001). Congeneric *Rhinogobius* species often strongly compete for habitats and food resources (Osugi et al., 1998; Takahashi et al., 2001; Sone et al., 2001; Sone et al., 2006). In Lake Erhai, *R. cliffordpopei* mostly use the littoral habitat (LH, water depth < 6 m). In contrast, *R. giurinus* mostly occupy the profundal habitat (PH, water depth ranging form 12 to 20 m, Guo et al., 2012). To the best of our knowledge however, no studies have investigated their trophic niche shifts and trophic relationship up to now. The aim of the present study was to understand how ontogeny and habitat use could affect the trophic relationship between two congeneric and invasive species by analyzing the trophic niche of two congeneric goby species using gut content analyses (GCA) and stable isotope analyses (SIA) in Lake Erhai. Specifically, we aimed to determine (i) how trophic niche shift during ontogeny for each species; (ii) whether the trophic niche in each habitat differs between species; and (iii) whether the trophic niche of each species differs between habitats.

Materials and methods

Study areas and habitat characteristics

Lake Erhai (105°5-17′ E, 23°35-58′ N) locates in Yunnan-Guizhou Plateau, southwest of China (Fig. 1). It is a freshwater lake with water surface area of 250 km^2 and maximum water depth of 21 m. There are twenty-eight fish species in recent year and most are planktivorous. The dominant fishes are non-native small-bodied species. *Rhinogobius cliffordpopei* and *R. giurinus* are the most abundant benthic fish species with an annual yield of 48 % of total fish yields (kg) in 2010.

The environmental characteristics, abundances of *R. cliffordpopei* and *R. giurinus*, and abundances of potential food resources in LH and PH were investigated seasonally in 2010 (Table 1).

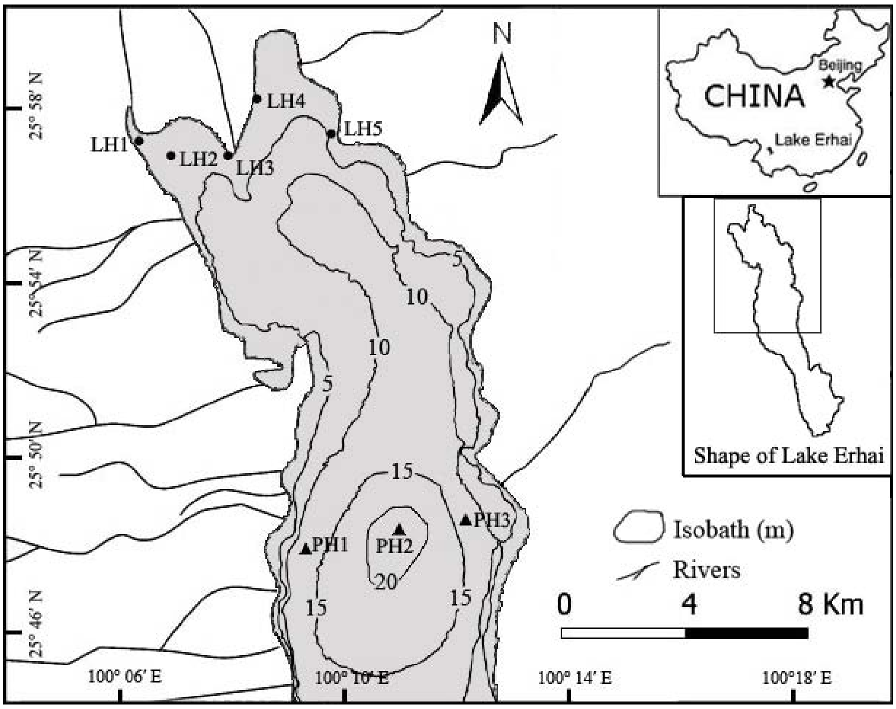

Fig. 1 Sampe sites of *R. cliffordpopei* in littoral habitat (●, LH1-LH5) and *R. giurinus* in profundal habitat (▲, PH1-PH3) in the central and northern sections of Lake Erhai (China) in 2010.

Field sampling

R. cliffordpopei and *R. giurinus* were collected monthly from May to August in 2010 using benthic fyke nets from five sample sites in LH (LH1-LH5) and three sample sites in PH (PH1-PH3, Fig. 1). The nets comprised a trunk stem with twenty traps (0.60 × 0.62 × 0.35 m for each one), two end-traps (1 m for each one) and two end-pockets (0.5 m each for each one). The stretched mesh size was 4mm. Four nets were deployed per site and per month from 8:00 to 12:00. After 24 hours, the nets were retrieved and all catches in the end pockets were collected. In each month, all *R. cliffordpopei* and *R. giurinus* in the same habitat were pooled and then sub-samples were taken for GCA and SIA. The sub-sample for GCA was preserved in 8 % formalin for two weeks and then transferred to 75% ethanol for storage. The sub-sample for SIA was stored at -20°C.

Table l Environmental characteristics, abundances of *R. cliffordpopei* and *R. giurinus*, and their potential food resources in littoral habitat (LH) and profundal habitat (PH) of Lake Erhai (China) in 2010. Values are mean ± standard deviation.

Parameters	Littoral zone habitat (LH)	Profundal zone habitat (PH)
Water depth (m)	3.1± 1.2 (n=40)	15.9 ± 3.1 (n=24)
Water temperature (°C)	16.9 ± 4.7 (n=40)	16.9 ± 4.6 (n=24)
Submerged macrophytes (g m^{-2})	3307 ± 881 (n=40)	0 (n=24)
pH	9.33 ± 0.32 (n=40)	9.08 ± 0.26 (n=24)
Secchi depth (cm)	214 ± 36 (n=40)	255 ± 25 (n=24)
Conductivity (μScm^{-1})	237 ± 17 (n=40)	234 ± 13 (n=24)
Dissolved oxygen (mg L^{-1})	9.23 ± 1.56 (n=40)	7.51 ± 0.88 (n=24)
Total nitrogen (mg L^{-1})	0.46 ± 0.12 (n=20)	0.43 ± 0.05 (n=12)
Total phosphorus (mg L^{-1})	0.0138 ± 0.0081 (n=20)	0.0142 ± 0.0069 (n=12)
Chlorophyll *a* (mg L^{-1})	8.27 ± 2.88 (n=20)	12.44 ± 3.09 (n=12)
R. cliffordpopei (ind. net^{-1} day^{-1})[1]	250 ± 156 (n=160)	37 ± 21 (n=96)
R. giurinus (ind. net^{-1} day^{-1})[1]	59 ± 28 (n=160)	318 ± 268 (n=96)
Macrozooplankton (ind. L^{-1})[2]	296 ± 61 (n=20)	93 ± 27 (n=12)
Tubificidae (ind. m^{-2})[3]	77 ± 29 (n=20)	145 ± 68 (n=12)
Aquatic insects (ind. m^{-2})[4]	102 ± 37 (n=20)	64 ± 25 (n=12)
Shrimp (ind. net^{-1} day^{-1})[5]	1981 ± 1245 (n=160)	2946 ± 1851 (n=96)

[1] Mean number of the two goby species collected per benthic fyke net in twenty-four. [2] Mean density of Cladocera (dominated by Daphniidae, Chydoridae and Bosminidae) and Copepoda (dominated by Cyclopoida); [3] Mean density of Tubificidae (dominated by *Limnodrilus*); [4] Mean density of aquatic insects (dominated by Chironomid); [5] Mean number of shrimp (dominated by Atyidae) collected per benthic fyke net in twenty-four hours.

Gut content analyses (GCA)

Total body length (L_T) and body mass (M_T) of each specimen for GCA were measured to the nearest mm and 0.01 g, respectively. Since there was no clear stomach for the two species, the fore-gut, which was defined as the section of intestine from the oesophagus to the posterior end of the first loop, was sampled for diet analyses (Xie et al., 2000; Xie et al., 2005). Fore-gut contents of non-empty guts were removed and preserved in 2 ml plastic tube with 75 % ethanol. Food items were identified to the lowest possible taxonomic level under a dissecting microscope. The weight of Cladocera and Ostracoda were calculated as 0.023 mg per individual. The weight of Copepoda was calculated as 0.014 mg per individual. The larva of Copepoda was calculated as 0.003 mg per individual (Zhang, 2005). The weight of fish eggs, plant materials and unidentified items were roughly equal to their volumes (specific gravity was assumed to be 1). After removing surface ethanol by blotting them on tissue paper, shrimp larvae, aquatic insects, Gastropoda and fish larvae were weighted to the nearest 0.1 mg (Hyslop, 1980). For each food category, frequency of occurrence (%O), percentage of number (%N), percentage of weight (%W) were calculated as: %O*i* = 100 O*i* / $\sum_1^n Oi$, where O*i* is the number of the guts that contain food category *i*), %N*i* = 100 N*i* / $\sum_1^n Ni$, where N*i* is the number of food category *i*) and %W*i* = 100 W*i* / $\sum_1^n Wi$, where W*i* is the weight of food category *i*) (Hyslop, 1980). Diet compositions of the two goby species were finally estimated using index of relative importance (%IRI) that was calculated as: %IRI = 100 IRI*i* / $\sum_1^n IRIi$, where IRI*i* = (%N*i* + %W*i*) / %O*i* (Assis, 1996).

Based on their life characteristics, specimens of the two species used for GCA were grouped into three life stages, i.e juveniles (gonados are at stage I, gonados are indistinguishable for sexes by naked eye), sub-adults (gonados are at stage II to III) and adults (gonados are at stage IV to V). In LH, L_T (mean ± SD, mm) of juveniles, sub-adults and adults for *R. cliffordpopei* were 14.7 ± 3.1 (n = 27), 27.4 ± 3.9 (n = 35) and 41.3 ± 6.2 (n = 41), respectively, and for *R. giurinus* were 15.4 ± 3.4 (n=34), 36.1 ± 2.9 (n=42) and 50.4 ± 6.4 (n=49), respectively. In PH, L_T of juveniles, sub-adults and adults for *R. cliffordpopei* were 15.1 ± 2.5 (n = 29), 29.9 ± 3.1 (n = 25) and 43.1 ± 5.6 (n = 41) respectively, and for *R. giurinus* were 14.9 ± 3.2 (n=37), 37.3 ± 5.7 (n=33), 53.4 ± 4.7 (n=30), respectively.

Stable isotope analyses (SIA)

To determine the isotope values of potential food resources in each habitat, macrozooplankton, Tubificidae, aquatic insects and shrimp larvae were sampled in May of 2010. Macrozooplankton were Cladocera (dominated by Daphniidae, Chydoridae and Bosminidae) and Copepoda (dominated by Cyclopoida). Tubificidae were dominated by *Limnodrilus* and aquatic insects were dominated by Chironomidae. Shrimp larvae were sampled from collections of benthic fyke nets and dominated by Atyidae (body mass was 75.5 ± 21.9 mg, mean ± SD). Macrozooplankton, Tubificidae and aquatic insects were kept alive in distilled water for 24 hours to clean gut contents, killed by freezing, and then subjected to SIA. Shrimp larvae were kept at -20°C. All the procedures were performed following the legislation of China (GB14925-2010).

Total body length (L_T) and body mass (M_T) of each specimen for SIA were measured to the nearest mm and 0.01 g, respectively. Dorsal muscles of each individual of gobies and shrimp larvae were used for SIA. Dorsal muscles of the individuals with the same L_T were pooled. These dorsal muscles and potential food resources (i.e. macrozooplankton, Tubificidae, aquatic insects and shrimp larvae) were oven dried at 60 °C for 48 hours and ground into homogeneous powders using a mortar and pestle. SIA were performed using an Elemental Analyzer (Flash EA1112 HT) along with an Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific，Inc., USA). Isotopic ratios were presented as relative to international standards (peedee belemnite carbonate (C) and atmospheric nitrogen (N)) in delta (δ) notation. Standard deviation (SD) of replicated sample was less than 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$. In LH, L_T (mean ± SD, mm) of juveniles, sub-adults and adults for *R. cliffordpopei* were 13.1 ± 4.0 (n = 10), 32.9 ± 3.6 (n = 12) and 41.8 ± 5.7 (n=13), respectively, and for *R. giurinus* were 14.4 ± 3.3 (n=14), 35.5 ± 4.5 (n=24) and 52.1 ± 8.4 (n=16), respectively. In PH, L_T of juveniles, sub-adults and adults for *R. cliffordpopei* were 13.6 ± 4.8 (n = 12), 30.2 ± 4.8 (n = 13) and 44.1 ± 6.4 (n = 10) respectively , and for *R. giurinus* were 14.2 ± 3.6 (n=10), 37.5 ± 6.4 (n=20) and 54.8 ± 8.5 (n=21), respectively.

Statistical analyses

Chi-square test (χ^2 test) was used to test the differences in diet compositions (%IRI) between life stages (Juveniles, Sub-adults and Adults) within species, and between species (*R. cliffordpopei* and *R. giurinus*) and habitats (LH and PH) within the same life stage.

The stable isotopic values of food resources showed no significant differences between LH and PH (Mann-Whitney tests, $P > 0.05$). After examination of normality and variances homogeneity of data using Kolmogorov- Smirnov and Levene’s tests, three-way analyses of variance (ANOVA) were used to test the differences of stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$ were the inverse transformed and tested separately) between life stages, species and habitats. If species and/or habitats showed significant effects on stable isotopic values, Mann-Whitney tests were used to test for the differences of stable isotopic values between species within habitat and/or between habitats within species at the same life stage.

The contributions of potential food resources to the diet of the two species were estimated using stable isotope mixing models (Stable Isotope Analysis in R; Parnell *et al*., 2008). Since there are no available trophic fractionation factor (TEF) values for the two goby species, we used an averaged TEF with a large SD as suggested by Inger *et al*. (2010). Here, the input TEF was 1.0 ± 1.0 ‰ (mean ± SD) for δ^{13}C and 3.3 ± 1.0 ‰ (mean ± SD) for δ^{15}N (Inger et al., 2010). Mean δ^{13}C and δ^{15}N values (±SD, n=3) of potential food resources in each habitat were used in the models. As life stages and habitats significantly affected the values of δ^{13}C and δ^{15}N, the models were run separately for different life stages in each habitat. Finally, the predicted percentage of contribution (mean ± SD) of each food source to the diet of the two species at the three life stages was averaged. All statistical analyses were conducted in R 2.14.2 (R Development Core Team, 2012).

Results

Ontogenetic trophic niche shift

Evidences from GCA

Juveniles of the two species showed significantly different diet compositions (%IRI) with sub-adults and adults (χ^2 test, $P < 0.05$), whereas diet compositions were similar between sub-adults and adults (χ^2 test, $P > 0.05$, Table 2). Specifically, %IRI of macrozooplankton was 50.2 - 71.0 % for juveniles but decreased to 20.4 - 35.5 % for sub-adults and 12.4 - 27.6 % for adults (Fig 2). %IRI of aquatic insects were less than 9.3 % for juveniles and increased to 20.4 - 55.7 % for sub-adults and 30.6 - 61.3 % for adults. %IRI of shrimp larvae ranged 8 - 19% and varied slightly with life stages.

Table 2 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) of *R. cliffordpopei* and *R. giurinus* between life stages (juveniles, sub-adults and adults) within species in littoral habitat (LH) and profundal habitat (PH) of Lake Erhai (China) in 2010.

			Juveniles			Sub-adults		
			χ^2	d.f.	*P*	χ^2	d.f.	*P*
LH	*R. cliffordpopei*	Sub-adults	21.09	9	0.012			
		Adults	38.04	9	<0.001	9.98	9	0.352
	R. giurinus	Sub-adults	33.47	9	<0.001			
		Adults	47.73	9	<0.001	11.59	9	0.237
PH	*R. cliffordpopei*	Sub-adults	33.47	9	<0.001			
		Adults	47.72	9	<0.001	16.35	9	0.060
	R. giurinus	Sub-adults	66.15	9	<0.001			
		Adults	79.73	9	<0.001	15.96	9	0.068

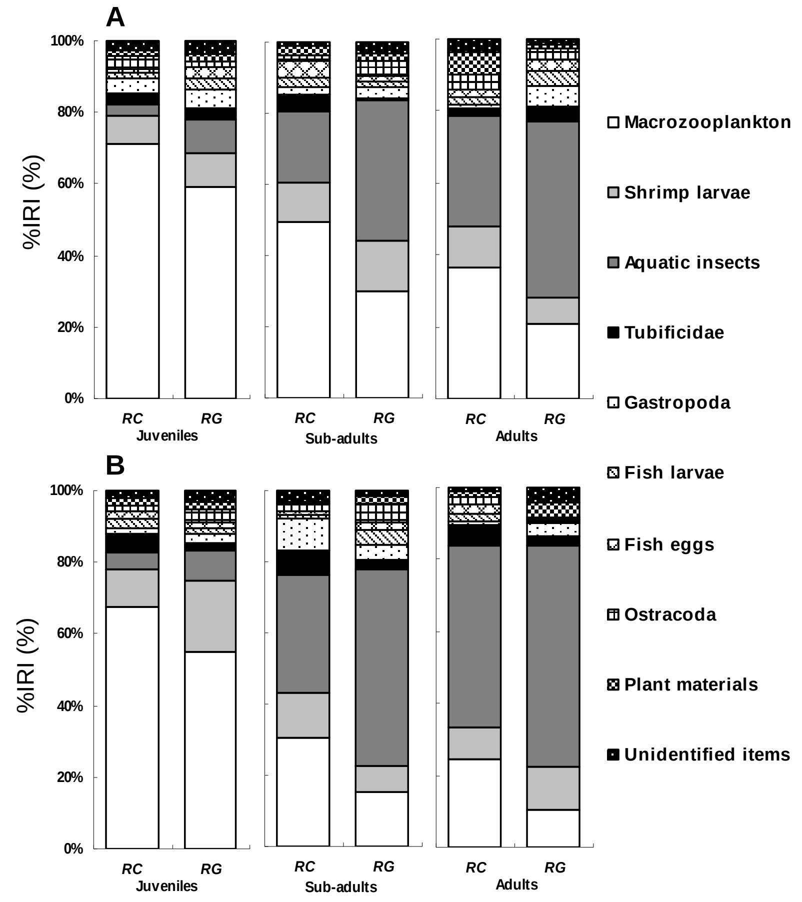

Fig. 2 Diet compositions (%IRI) of *R. cliffordpopei* (*RC*) and *R. giurinus* (*RG*) at three life stages (juveniles, sub-adults and adults) in (A) littoral habitat and (B) profundal habitat of Lake Erhai (China) in 2010.

Evidences from SIA

The life stages had significant effects on δ^{13}C and δ^{15}N values (three-way ANOVA, $P < 0.001$, Table 3). Both species generally showed steadily depleted δ^{13}C and δ^{15}N from juveniles to adults (Fig 3). The contribution of macrozooplankton was highest (43.4 - 74.1 %) for juveniles and lowest for adults (4.2 - 7.4 %, Fig 4). Aquatic insects and Tubificidae contributed little (3.4 - 9.2 %) to diets of juveniles but they contributed 59.5 - 81.9 % and 89.9 - 90.14 % to diets of sub-adults and adults, respectively (Fig 4).

Table 3 Three-way analysis of variances (ANOVA) used to test the differences in stable isotopic values (δ^{13}C and δ^{15}N, the inverse transformed) between life stages (juveniles, sub-adults and adults), species (*R. cliffordpopei* and *R. giurinus*) and habitats (littoral habitat (LH) and profundal habitat (PH)) in Lake Erhai (China) in 2010.

	Sources	SS	d.f.	MS	*F*	*P*
^{13}C (‰)	Life stages	1.11E-3	2	5.57E-4	321.63	<0.001
	Species	3.74E-5	1	3.744E-5	21.73	<0.001
	Habitats	8.74E-5	1	8.74E-5	50.48	<0.001
	Life stages × Species	1.10E-6	2	5.001E-7	0.31	0.730
	Life stages × Habitats	1.16E-6	2	8.00E-7	0.46	0.230
	Species × Habitats	1.25E-5	1	1.25E-7	7.24	0.008
	Life stages × Species × Habitats	7.22E-6	2	3.10E-6	2.26	0.111
	Residuals	2.74E-4	158	1.70E-6		
δ^{13}N (‰)	Life stages	4.87E-2	2	2.43E-2	59.43	<0.001
	Species	2.97E-3	1	2.97E-3	7.23	0.008
	Habitats	0.12	1	0.12	301.98	<0.001
	Life stages × Species	5.50E-5	2	2.801E-5	0.67	0.510
	Life stages × Habitats	7.900E-5	2	3.950E-5	1.62	0.204
	Species × Habitats	4.83E-3	1	4.83E-3	11.79	<0.001
	Life stages × Species × Habitats	2.40E-4	2	1.20E-4	0.29	0.750
	Residuals	0.06	158	4.10E-4		

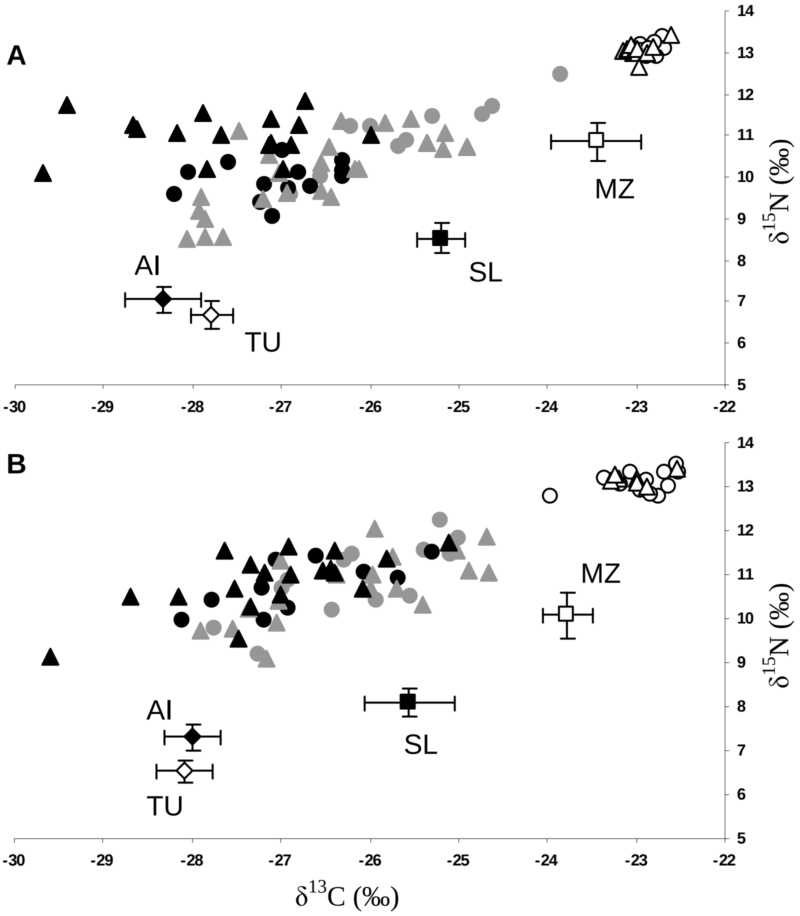

Fig. 3 Biplot of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of *R. cliffordpopei* (juveniles (○), sub-adults (○) and adults (●)), *R. giurinus* (juveniles (Δ), sub-adults (Δ) and adults (▲)), and their potential food resources (mean ± standard deviation, n=3) in littoral habitat (A) and profundal habitat (B) of Lake Erhai (China) in 2010. The potential food resources are macrozooplankton (MZ), shrimp larvae (SL), aquatic insects (AI) and Tubificidae (TU).

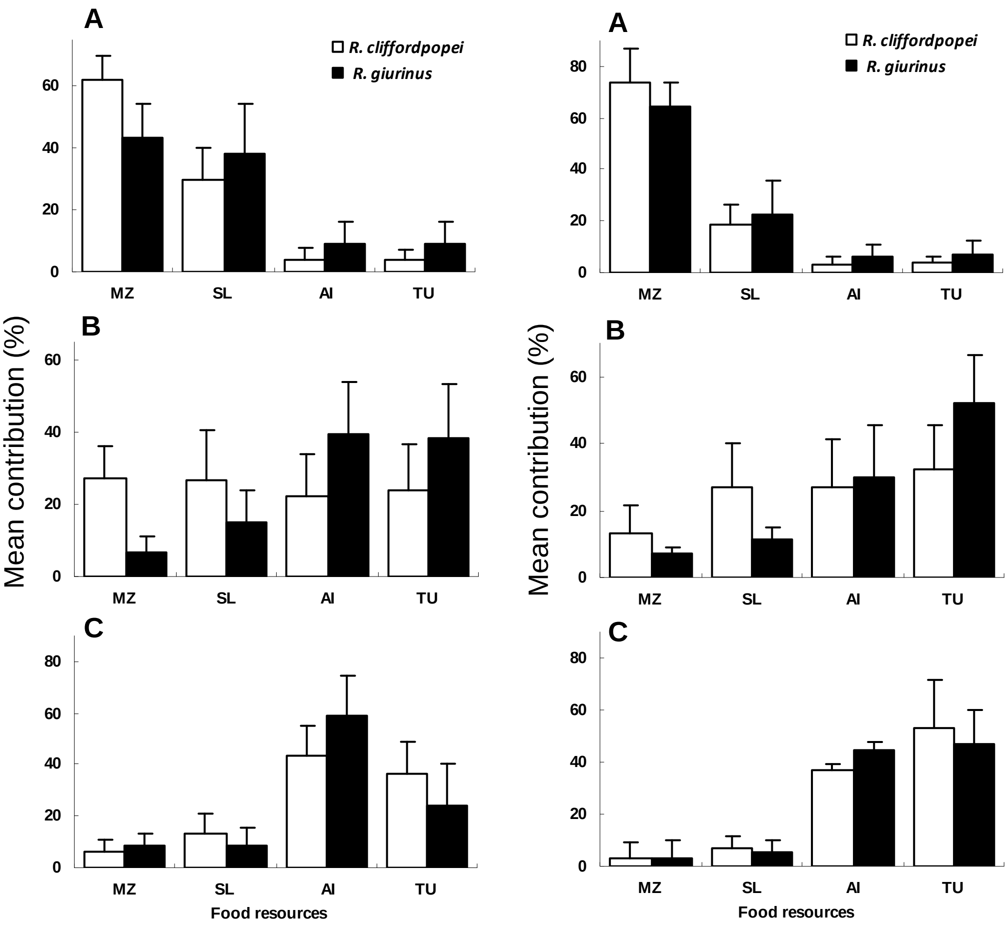

Fig. 4 The contributions (mean ± SD, %) of potential food resources to diets of *R. cliffordpopei* and *R. giurinus* at three life stages (A: juveniles, B: sub-adults and C: adults) in (left) littoral habitat and (right) profundal habitat of Lake Erhai (China) in 2010. The values were caculated using stable isotope mixing models. Potential food resources were macrozooplankton (MZ), shrimp larvae (SL), aquatic insects (AI) and Tubificidae (TU). Bars were standard deviation of each value.

Trophic niche differences between species within habitats

Evidences from GCA

In LH and PH, food compositions (%IRI) of the two species were similar for juveniles (χ^2 test, $P > 0.05$) but significantly different for sub-adults and adults (χ^2 test, $P < 0.05$, Table 4 and Fig 2). Sub-adults and adults of *R. cliffordpopei* preyed more macrozooplankton than *R. giurinus*, while *R. giurinus* consumed more aquatic insects than *R. cliffordpopei* in both habitats (Fig 2).

Evidences from SIA

Species significantly affected $\delta^{13}C$ and $\delta^{15}N$ of these two species (three-way ANOVA, $P < 0.05$, Table 3). In LH, $\delta^{13}C$ and $\delta^{15}N$ of juveniles were similar between the two species (Mann-Whitney, $P > 0.05$), but sub-adults of *R. cliffordpopei* showed more enriched $\delta^{13}C$ and $\delta^{15}N$ than *R. giurinus.* Adults of *R. cliffordpopei* were more depleted in $\delta^{15}C$ but enriched in $\delta^{15}N$ than *R. giurinus* (Mann-Whitney, $P < 0.05$, Table 5 and Fig 3). Macrozooplankton and shrimp larvae contributed more to diets of sub-adults in *R. cliffordpopei* in LH, whereas aquatic insects and Tubificidae contributed more to diets of sub-adults and adults of *R. giurinus* (Fig 4). In PH, the two species showed similar $\delta^{13}C$ and $\delta^{15}N$ at each life stage (Mann-Whitney, $P > 0.05$, Table 5, Fig 3 and Fig 4).

Table 4 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) between *R. cliffordpopei* and *R. giurinus* within habitat (littoral habitat (LH) and profundal habitat (PH)) at the same life stages (juveniles, sub-adults and adults) in Lake Erhai (China) in 2010.

		R. giurinus								
		Juveniles			Sub-adults			Adults		
	R. cliffordpopei	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
LH	Juveniles	6.07	9	0.732						
	Sub-adults				19.01	9	0.025			
	Adults							18.19	9	0.032
PH	Juveniles	8.10	9	0.523						
	Sub-adults				22.03	9	0.008			
	Adults							19.19	9	0.024

Trophic niche differences between habitats within species

Evidences from GCA

Diet compositions (%IRI) of the two species were similar between LH and PH for juveniles (χ^2 test, $P > 0.05$) but significantly different for sub-adults and adults (χ^2 test, $P < 0.05$, Table 6 and Fig 2). Sub-adults and adults generally preyed more macrozooplankton but less aquatic insects in LH than in PH (Fig 2).

Evidences from SIA

Habitats showed significant effects on $\delta^{13}C$ and $\delta^{15}N$ of the two species (three-way ANOVA, $P < 0.001$, Table 3). Specifically, $\delta^{13}C$ and $\delta^{15}N$ of *R. cliffordpopei* were similar at the three life stages between habitats (Mann-Whitney, $P > 0.05$), whereas *R. giurinus* displayed more enriched δ^{13}C and depleted δ^{15}N in LH than in PH at sub-adults and adults (Mann-Whitney, $P \geq 0.05$, Table 7). Sub-adults and adults of *R. giurinus* consumed more aquatic insects but less Tubificidae in LH than in PH (Fig 4).

Table 5 Mann-Whitney tests used to test the differences in stable isotopic values (δ^{13}C and δ^{15}N) between *R. cliffordpopei* and *R. giurinus* within habitat (littoral habitat (LH) and profundal habitat (PH)) at the same life stages (juveniles, sub-adults and adults) in Lake Erhai (China) in 2010.

			R. giurinus					
			Juveniles		Sub-adults		Adults	
		R. cliffordpopei	W	*P*	W	*P*	W	*P*
LH	δ^{13}C (‰)	Juveniles	117	0.289				
		Sub-adults			155	<0.001		
		Adults					45	0.010
	δ^{13}N (‰)	Juveniles	119	0.239				
		Sub-adults			144	0.003		
		Adults					48	0.015
PH	δ^{13}C (‰)	Juveniles	92	0.640				
		Sub-adults			52	0.341		
		Adults					174	0.229
	δ^{13}N (‰)	Juveniles	93	0.634				
		Sub-adults			69	0.435		
		Adults					98	0.353

Table 6

Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) between habitats (littoral habitat (LH) and profundal habitat (PH)) within species at the same life stages (juveniles, sub-adults and adults) in Lake Erhai (China) in 2010.

		LH								
		Juveniles			Sub-adults			Adults		
	PH	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
R. cliffordpopei	Juveniles	2.89	9	0.968						
	Sub-adults				18.15	9	0.033			
	Adults							18.78	9	0.027
R. giurinus	Juveniles	6.56	9	0.682						
	Sub-adults				19.75	9	0.020			
	Adults							19.26	9	0.023

Table 7

Mann-Whitney tests used to test the differences in stable isotopic values (δ^{13}C and δ^{15}N) between habitats (littoral habitat (LH) and profundal habitat (PH)) with species at the same life stages (juveniles, sub-adults or adults) in Lake Erhai (China) in 2010.

			LH					
			Juveniles		Sub-adults		Adults	
		PH	W	*P*	W	*P*	W	*P*
R. cliffordpopei	δ^{13}C (‰)	Juveniles	112	0.154				
		Sub-adults			41	0.245		
		Adults					143	0.378
	δ^{13}N (‰)	Juveniles	93	0.093				
		Sub-adults			36	0.356		
		Adults					120	0.289
R. giurinus	δ^{13}C (‰)	Juveniles	131	0.202				
		Sub-adults			31	<0.001		
		Adults					19	<0.001
	δ^{13}N (‰)	Juveniles	49	0.672				
		Sub-adults			27	<0.001		
		Adults					23	<0.001

Discussion

Diet composition and ontogenetic trophic niche shift

Both gut content analyses (GCA) and stable isotope analyses (SIA) demonstrated that macrozooplankton (Cladocera and Copepoda) were main diets for juveniles and also important for sub-adults of these two goby species in Lake Erhai. Our findings were consistent with those observed in native lakes along the middle and lower reaches of the Yangtze River including Liangzi Lake (Xie et al., 2005) and Biandantang Lake (Xie et al., 2000; Zhang, 2005). When the individulas grow into sub-adults and adults, their trophic niche showed a strong ontogenetic shift whereby they gradually shifted diets to aquatic insects and Tubificidae. As substrata-associated species, most gobies mainly consume macrozoobenthos when they settle to bottom after pelagic stages (Sone et al., 2001; Kakareko et al., 2005; Grabowska et al., 2009; Borza et al., 2009). Among those macrozoobenthos, Chironomidae larvae are one of the most important preys for species of the genus *Rhinogobius* (Xie et al. 2000; Sone et al., 2001; Xie et al. 2005; Zhang 2005; Rusuwa et al., 2009). Some other goby species have also shown a clear ontogenetic diet shift (Kanou et al., 2005; Rusuwa et al., 2009). In the

Ado River of Japan, for instance, the three most important preys of *Rhinogobius brunneus* ‘orange’ for juveniles are Ephemeroptera, Diptera and Cyanophyta but are Ephemeroptera, Trichoptera and Detritus for adults (Rusuwa et al., 2009).

In the present study, the contribution of Tubificidae to diets of sub-adults and adults was much higher using SIA than GCA. Similarly, GCA revealed that the two gobies consumed a few Tubificidae in the native lakes (Xie et al., 2000; Xie et al., 2005; Zhang, 2005). Those differences probably results from the easy digestibility and difficult detectability of Tubificidae since GCA is usually more vulnerable to bias due to the differences in digestibilitya, delectability and quantifiability among food items compared with SIA technique (Polito et al., 2011; Inger et al., 2010; Cucherousset et al., 2012). Moreover, GCA and SIA found that shrimp larvae contributed much more to diets of the two species in Lake Erhai than in the native lakes (Xie et al., 2000; Zhang, 2005). Indeed, many goby species have been observed to prey shrimp larvae (e.g. Amphipoda), such as round goby *Neogobius melanostomus* (Pallas, 1814) and bighead goby *Neogobius kessleri* (Günther, 1861) in River Danube of Hungary (Borza et al, 2009; Polačik et al., 2009), monkey goby *Neogobius fluviatilis* (Pallas, 1814) and racer goby *Neogobius gymnotrachelus* (Kessler, 1857) in River Vistula of Poland (Kakareko et al., 2005). Highly abundant shrimps in Lake Erhai can provide large number of shrimp larvae for them, whereas shrimps resources are far less in the native lakes than Lake Erhai (Qin et al., 2005).

Trophic niche differences between species within habitat

In littoral habitat (LH), small-sized juveniles of the two gobies showed similar trophic niche but medium-sized sub-adults and large-sized adults displayed different trophic niches. The body size of fishes has a substantial effect on trophic relationships because it is a pivotal determinant affecting individual’s dispersal abilities, mortality risks (Einum et al., 2006), foraging efficiencies and/or competitive abilities (Hjelm and Persson, 2001; Colloca et al., 2010; Nakayama and Fuiman, 2010). Small individuals at early ontogeny are small-sized, gape-limited and thus are almost exclusively pelagic and zooplanktivorous. Although they may strongly compete for a limited food resource during this period, such a competition rarely results in trophic niche specialization (Nakayama and Fuiman, 2010; Colloca et al., 2010). In later ontogenetic life stages, however, fishes show much broader prey size spectrums of diets. Moreover, large-sized individuals may have better locomotor capacity and higher feeding efficiency (Hjelm and Persson, 2001; Borcherding et al., 2010). Therefore, they are able to exploit different diets and thus can specialize and partition food resources, which may allows a coexistence rather than a competitive exclusive among closely-related or ecological similar species within a community (Colloca et al., 2010; Fanelli et al., 2011, Davis et al., 2012). Several studies have demonstrated that goby species (sub-adults or adults) exhibited food resources partitioning when they become abundant out of native ranges (Kakareko et al., 2005; Borza et al., 2009; Borcherding et al., 2012). The River Danube of Hungary, for instance, bighead goby mainly consumed *Dikerogammarus* spp but round goby preferred Chironomidae larvae in spring (Borza et al., 2009).

In profundal habitat (PH), SIA indicated that sub-adults and adults showed similar trophic niche between species, whereas their diet composition was different by GCA. Since GCA provides a snapshot of diets reflecting an individual's recent and opportunistic diets but SIA is an integrative tool to elucidate long-term dietary records (Post, 2002; Polito et al., 2011; Cucherousset et al., 2012), the results of SIA were probably more reliable. Thus, sub-adults and adults of the two species displayed different patterns of food resources partitioning between LH and PH. Dietary resource exploitations among competing fishes may vary with environmental factors including habitat conditions (e.g. stable *vs* highly variable ecosystem, Davis et al., 2012), prey availabilities (Shimose et al., 2010), density of competitors (Byström and García-Berthou, 1999; Kaspersson et al., 2010), shape of food patches and group size of competitors (Kim and Grant, 2007). In Lake Erhai, LH and PH show a big difference in habitat conditions (e.g. water depths and densities of submerged macrophytes), food abundances, densities of competitors and predators, etc. (Table 1, Guo et al., 2012). Further investigations are needed to elucidate the specific effects of those factors on patterns of competition and/or food partitioning between the two species in Lake Erhai.

Trophic niche differences between habitats within species

Invasive gobies in many cases show an opportunistic feeding strategy when the available food supply vary with habitats (Kakareko et al., 2005; Grabowska et al., 2009; Polačik et al., 2009; Rusuwa et al., 2009) and/or times (e.g. months (Borcherding et al., 2012) or seasons (Xie et al., 2000; Grabowska et al., 2009; Rusuwa et al., 2009). Food items of gobies of the genus *Rhinogobius* are the wide spectrum of potential food, including Tubificidae, Mollusca (e.g. snails, bivalves, etc.), Crustacea (e.g. zooplankton, shrimp (Decapoda), etc.), aquatic insects (e.g. Baetidae, Leptophlebiidae, Chironomidae, etc), as well as shrimp or fish eggs and small fishes (Xie et al. 2000; Sone et al., 2001; Xie et al. 2005; Zhang 2005; Rusuwa et al., 2009). In our study, juveniles of the two gobies did not exhibited clear differences in diet compositions between LH and PH due largely to their exclusively zooplanktivorous. However, their sub-adults and adults generally consumed more macrozooplankton and Chironomidae larvae in LH but more Tubificidae and aquatic insects in PH. In Liangzi Lake (a small shallow lake along the middle reach of the Yangtze River), Xie et al. (2005) also revealed the *R. giurinus* ate more Cladocera and aquatic insects in the central area (i.e. farther than 1.5 km to the nearest shoreline, deeper water depth but less submerged macrophytes) than those in the near-shore area. In Lake Erhai, the density of macrozooplankton and aquatic insects in LH is almost twice as high as in PH, whereas the Tubificidae is much more abundant in PH than LH (Table 1). The diets of sub-adults and adults seem to closely match the abundances of available food resources in the two habitats. Similar patterns were observed in monkey goby and racer goby in River Vistula of Poland where they predominantly consumed Chironomidae larvae in main channels but Mollusca and Oligochaeta in reservoir because available Chironomidae larvae biomass was almost 30 times less in the main channel than in the reservoir (Kakareko et al., 2005). The plastic and opportunistic feeding strategy is one of important biological traits allowing them to be successful invaders since food resources are usually different from what it is in natural ranges (Grabowska et al., 2009; Borcherding et al., 2012; Kornis et al., 2012).

Conclusively, the present study demonstrated that these two goby species strongly shifted trophic niche from juveniles to adults. They exploited similar food resources at juveniles but different ones at sub-adults and adults within habitat. Moreover, these two species showed a plastic and opportunistic feeding strategy when available food supply vary with habitats. However, the competing behavior underpinning such outcomes is particularly interesting but our study poorly understood it. Further investigations, therefore, are suggested to examine how behaviors of exploitive competition (e.g. food intake, feeding rate) or interference competition (e.g. chase rate, attack rate, food holding capacity) varies with habitat conditions such as food resource levels, population densities, predation risks, etc.

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Age, growth and population dynamics of two congeneric and invasive goby species: the implications for management

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Abstract

Many goby species have become widely established outside of their native ranges, yet little is known of an effective and efficient management strategy giving a full consideration to their life characteristics. In present study, the age, growth and population dynamics of two highly abundant and introduced goby species (*Rhinogobius giurinus* and *Rhinogobius cliffordpopei*) were investigated in Lake Erhai (China) with the aim at improving the current management strategies. The results demonstrated that those two gobies are short-lived species with a life span of one year. *R. cliffordpopei* showed the highest body length in March and lowest June or July, while *R. giurinus* showed the highest body length in June and lowest in August. The von Bertalanffy growth function was $L_t = 55.65\,[-\,e^{-0.58\,(t\,+\,1.48)}]$ for *R. cliffordpopei* and $L_t = 68.25\,[-\,e^{-0.99\,(t\,+\,0.78)}]$ for *R. giurinus*. The total mortality, natural mortality, fishing mortality and exploitation rate was 2.12, 0.85, 1.25, 0.59 for *R. cliffordpopei*, and 1.73, 1.17, 0.56, 0.32 for *R. giurinus*, respectively. The *CPUE* of benthic fyke nets was low from April to June for *R. cliffordpopei* and from July to September for *R. giurinus*, whereas it was high from July to September for *R. cliffordpopei* and from April to June, September to November for *R. giurinus*. These life characteristics are highly recommended to be incorporated into the management strategies of the two species. Consequently, several specific suggestions are made to improve the cost-efficiency of current management programs.

Key words: gobies, biological invasion, growth, population dynamics, invasive species management

Introduction

Invasive alien species are one of the greatest threats to global biodiversity, global environmental change, socio-economic development and/or human health (Butchart *et al*., 2010; Vörösmarty *et al*., 2010; Cucherousset & Olden, 2011). Fishes are among the most introduced group of aquatic animal (García-Berthou *et al*., 2005; Gozlan, 2008). The overall number of freshwater fishes introduced from known sources reaches 624 and the rate of non-native freshwater fishes has doubled in the past 30 years (Gozlan, 2008; Gozlan et al., 2010). Since many invasive fishes show significant ecological, evolutionary and/or economic impacts on recipient ecosystems, several measures (e.g. physical removal, chemical eradication, bio-manipulation, etc.) have been widely developed and implemented to control, contain or eradicate a wide range of invasive fish species (Taylor & Hastings, 2004; Britton et al., 2011). The effectiveness and efficiency of management strategies are often highly dependent upon the life traits of the target invasive species (Ludgate & Closs, 2003; Britton *et al*., 2011). As a consequence, understanding the life characteristics of established population is a fundamental prerequisite and imperative need for developing effective management strategies.

Watersheds of the Yunnan Province have been one of the highest biodiversity hotspots in China (Xie *et al*., 2001). However, many invasive fish species have been introduced since the 1950-60s and a large number of native fish species (about one third of 432 native fish species) have become endangered or extinct (Xie *et al*., 2001; Yuan *et al*., 2010). For instance, seventeen native fish species were present in Lake Erhai in the 1950s (Du & Li, 2001). Currently, there are only seven native species coexisting with twenty-two introduced species. The most notorious introduced fish species in lakes of the Yunnan Province are small-bodied goby of genus *Rhinogobius, Neosalanx taihuensis, Pseudorasbora parva, Hypseleotris swinhonis, Hemiculter leucisculus* and *Abbottina rivularis* (Yuan *et al*., 2010). Although significant attention has been paid to these species, the control actions have minimal success or require indefinite investments and thus the outcomes are often discouraging (Du & Li, 2001; Yuan *et al*., 2010). An important fact overlooked is that the most of these management strategies are implemented without an appropriate knowledge of life characteristics.

Freshwater gobies of the genus *Rhinogobius* (Gill 1859) are the dominant benthic fish fauna in most East Asian countries, including China, Korea, Japan and some other regions of south-eastern Asia, such as Philippines, Vietnam and Thailand (Chen & Shao, 1996; Sone *et al*., 2001; Wu & Zhong, 2008). In China, there are at least 17 nominal species of *Rhinogobius*. *Rhinogobius giurinus* (Rutter, 1897) and *Rhinogobius cliffordpopei* (Nichols, 1925) are frequent small fishes in the middle and lower reaches of the Yangtze River, especially in shallow and macrophytic lakes (Wu & Zhong, 2008). The two species were inadvertently introduced into most lakes of the Yunnan Province in the 1950-60s and introduced simultaneously to Lake Erhai in 1961 (Du & Li, 2001, Xie *et al*., 2001; Yuan *et al*., 2010). Since then they have become the most dominant benthic fish species with annual yield accounting for 48 % of total fish yields (kg) of the lake in 2010. In native lakes along the middle and lower reaches of the Yangtze River, they are ecologically similar species with comparable life histories, such as small-bodied (maximal total body length < 80 mm), reproducing at spring and early summer, macrozooplanktivorous, etc. (Xie *et al.*, 2000; Xie *et al.*, 2005; Zhang, 2005). In Lake Erhai, however, our previous study demonstrated that ecological habits of these two gobies are highly plastic and show several different aspects with the populations in native ranges. There were also differences between those two species in terms of habitat preferences (Guo *et al*., 2012a) and reproductive biology (Guo *et al*., 2012b). Currently, the main management strategy in the lake is the local fishery by benthic nets for the two species. This strategy pays little attention to the life characteristics of the two species and did not give any consideration to possible biological differences between them. As a consequence, the present study investigated the age, growth and population dynamics of those two goby species. The possible differences between them are tested too. The findings aim at improving the current management strategy and developing a species-specific management program for the two species in the lake.

Materials and Methods

Study sites

This study was carried out in Lake Erhai (105°5-17′ E, 23°35-58′ N), a shallow freshwater lake in Yunnan-Guizhou plateau in southwest China (Fig. 1). Its water surface area is about 250 km^2 with a catchment area of 2,600 km^2. The maximal water depth is 21 m. The dominant non-native species are freshwater goby *R. giurinus* and *R. cliffordpopei*, *Neosalanx taihuensis* (Chen 1965), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873) and *Hemiculter leucisculus* (Basilewsky, 1855). As the most abundant benthic fishes (the annual yield is about 48 % of total fish yields (kg) in the lake), *R. giurinus* and *R. cliffordpopei* show strongly habitat segregation, i.e. *R. cliffordpopei* mainly uses littoral habitat (LH, water depths less than 6 m, high abundance of submerged macrophytes, submersed-macrophyte detritus substrata) and *R. giurinus* mainly uses profundal habitat (PH, water depth range from 12 to 20 m, no submerged macrophytes, silt and coarse sand substrata, Guo et al., 2012a). Therefore, *R. cliffordpopei* were collected in five sites in LH (LH1-LH5) and *R. giurinus* were collected in three sites in PH (PH1-PH3, Fig. 1).

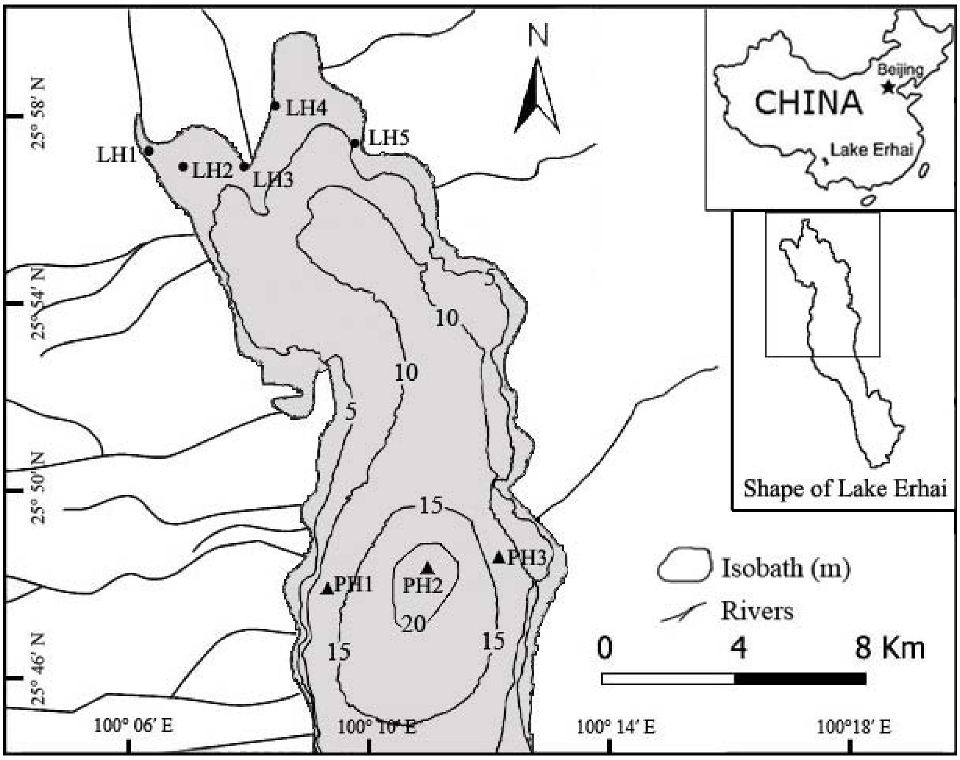

Fig. 1 Sample sites of *R. cliffordpopei* in littoral habitat (●, LH1-LH5) and *R. giurinus* in profundal habitat (▲, PH1-PH3) in the central and northern parts of Lake Erhai (China).

Fish sampling and data collection

These two gobies were caught monthly using fyke nets with stretched mesh size of 4 mm. Total length of the net was 15 m, including 12 m of traps, 2 m of end traps and 1 m of end pockets (Guo et al., 2012a). In the early of each month from October

2009 to October 2011, four nets were deployed per sampling site at 8:00 to 12:00 with a stone in the each end of nets to fix them at bottom. After 24 hours, all fishes in end pockets of the nets were collected and identified to the species level. In each month, we pooled all individuals of *R. cliffordpopei* from the five sample sites in LH and *R. giurinus* from the three sample sites in PH. The random sub-samples (the individuals range 400-800 for each species) from the pool were took and kept in a refrigerator for later analysis.

The individuals of the sub-samples were counted and measured the total body length (L_T) and body mass (M_T) to the nearest mm and 0.01 g, respectively. In each month, the scales of thirty individuals were sampled at random to determine the age. The sexes of about 200-300 individuals from the sub-samples were determined based on the macroscopic appearances of gonads (Guo *et al*., 2012b). The scales were immersed in 10 % NaOH for three hours, cleaned by running water and then read under an optical microscope using the criteria of Steinmetz & Müller (1991).

Data calculation and statistical analyses

Monthly body length-frequency was used to analyze the cohort structures. In the same month, Kolmogorov-Smirnov (K-S) test was used to test the differences in body length-frequency between males and females within species, and between species for the entire populations (including males, females and those unidentifiable for sexes).

Growth patterns were first estimated using monthly changes in mean L_T and body condition. Body condition was the *b* estimated by a linear regression between L_T and M_T: $\log_{10} M_T = a + b \log_{10} L_T$ (Froese, 2006). The mean L_T and body condition were compared between males and females within species, and between species for the entire populations using Mann-Whitney test and analyses of covariance (ANCOVAs) with L_T as a covariate, respectively. Second, growth patterns were described by von Bertalanffy growth function (Von Bertalanffy, 1938): $L_t = L_\infty [- e^{-K(t - t_0)}]$, where L_t is the total body length at the time t, L_∞ is the asymptotic length, K is the von Bertalanffy growth coefficient, t_0 is the theoretical age at $L_T = 0$. VBGF was only estimated for the entire populations because the sexes of most individuals were almost unidentifiable by macroscopic appearances of gonads in five months of a year. The function parameters were estimated using Electronic Length Frequency Analysis (ELEFAN) in the FiSAT II package (FAO – ICLARM Fish Stock Assessment Tools, Version 1.2.2, Gayanilo *et al.*, 2005). t_0 is was calculated as $\log_{10}(-t_0) = -0.392 - 0.275 \log_{10} L_\infty - 1.038 \log_{10} K$ (Pauly, 1979).

Population dynamics of the two species were described using mortality, mean size at first sexual maturity (L_{50}), mean time at first sexual maturity (T_{50}), maximum longevity (T_m) and catch per unit effort (*CPUE*) of the fyke nets. Specifically, mortality calculations were conducted using the procedures provided by the FiSAT II package (Gayanilo *et al.*, 2005). Total mortality (*Z*, *i.e.* the sum of natural and fishing mortality) was estimated using length-converted catch curves (Pauly, 1983). This method pools a long series of samples that represent a steady-state population and generate a single frequency distribution accounting for their relative importance. *Z* is then calculated on the descending part of this single global distribution (Pauly, 1983). Natural mortality (*M*) is correlated with asymptotic length (L_∞), the von Bertalanffy growth coefficient (*K*), and mean environmental temperature (*T*) by the Pauly's *M* equation (Pauly, 1987): $\ln(M) = -0.015 - 0.279 \ln(L_\infty) + 0.654 \ln(K) + 0.463 \ln(T)$. The mean annual temperature used for M estimates was 18.7 °C. It was averaged from the monthly temperatures of the Lake from October 2009 to October 2011. Fishing mortality (*F*) was then obtained by subtracting *M* from *Z*. The exploitation ratio (*E*) was calculated as $E = F/Z$. L_{50} is defined as the L_T at which 50% of the individuals are at an advanced maturation stage (i.e. gonads are at stage IV and V; Guo *et al.*, 2012b). It was quantified using logistic regression models with binomial maturity data (immature 0, mature 1) at any L_T of the entire populations. T_{50} was determined as: $T_{50} = \{-\ln[1 - (L_{50} L_\infty^{-1})] K^{-1}\} + t_0$ (García *et al.*, 2009). The maximum longevity (T_m) was calculated as $\log_{10}(T_m) = 0.549 + 0.957 \log_{10}(T_{50})$ (Froese & Binohlan, 2000; García *et al.*, 2009). Finally, catch per unit effort (*CPUE*, $ind.\ net^{-1}\ day^{-1}$) of the fyke nets was calculated monthly, which was defined as the number of goby species caught in one net per day. Differences of *CPUE* (log-transformed) between species and months were tested using repeated-measures ANOVA (RM-ANOVA) with species as a fixed factor and months as a random factor. Sphericity assumption was tested using Mauchly's test and the degrees of freedom were adjusted by Greenhouse–Geisser Epsilon when data violated the assumption of sphericity. For the same month, Mann-Whitney was subsequently used to test the differences in *CPUE* between species if species have significant effects on *CPUE*. The same approaches were used to test the differences of *CPUE* between sexes (fixed factors) within species and months (random factors).

The normality and variances homogeneity of data were tested using K-S test and Levene’s tests. If not mentioned otherwise, statistical analyses were performed using R version 2.14.2 (R Development Core Team, 2012).

Results

Over the study period, a total of 14,462 individuals of *R. cliffordpopei* and 16,793 individuals of *R. giurinus* (Fig. 4) were measured. From November to May, 3,935 individuals were identified for sex in *R. cliffordpopei* (2,296 males and 1,639 females (Fig. 2)). From January to July, 3,297 individuals were identified for sex in *R. giurinus* (1,690 males and 1,607 females (Fig. 3)).

Age and cohort structures

The age and cohort structures analyses indicated that the life span of two species is about one year. Specifically, most of the scales examined were readable for annuli (72.1% for *R. cliffordpopei* and 75.8 % for *R. giurinus*). The ages were 0+ for all those read scales.

For males and females of *R. giurinus*, the body length-frequency analyses demonstrated that there was a single cohort from January to July in 2010 (the cohort was born in 2009) and another one in 2011 (the cohort was born in 2010) (Fig. 2). Males and females showed significantly different body length-frequency in December 2009, January and April 2010, November and March 2011 (K-S test, $P < 0.05$, Table I). Similarly, both sexes of *R. giurinus* showed a single cohort from January to July in 2010 and another one 2011 (Fig. 3). They displayed similar body length-frequency between sexes except April 2010 (K-S test, $P > 0.05$, Table 1). Three cohorts were identified for the entire populations of the two species (Fig. 4). For *R. cliffordpopei*, the cohort born in 2009, 2010 and 2011 was found from October 2009 to May 2010, April 2010 to June 2011, and April 201l to October 2011, respectively (Fig. 4). In *R. giurinus*, the cohort born in 2009, 2010 and 2011 was found from October 2009 to July 2010, July 2010 to August 2011, and July 201l to October 2011, respectively (Fig. 4). Body length-frequency between the two species was significantly different from December 2009 to October 2010 and from April to October 2011(K-S test, $P < 0.001$,

Table I).

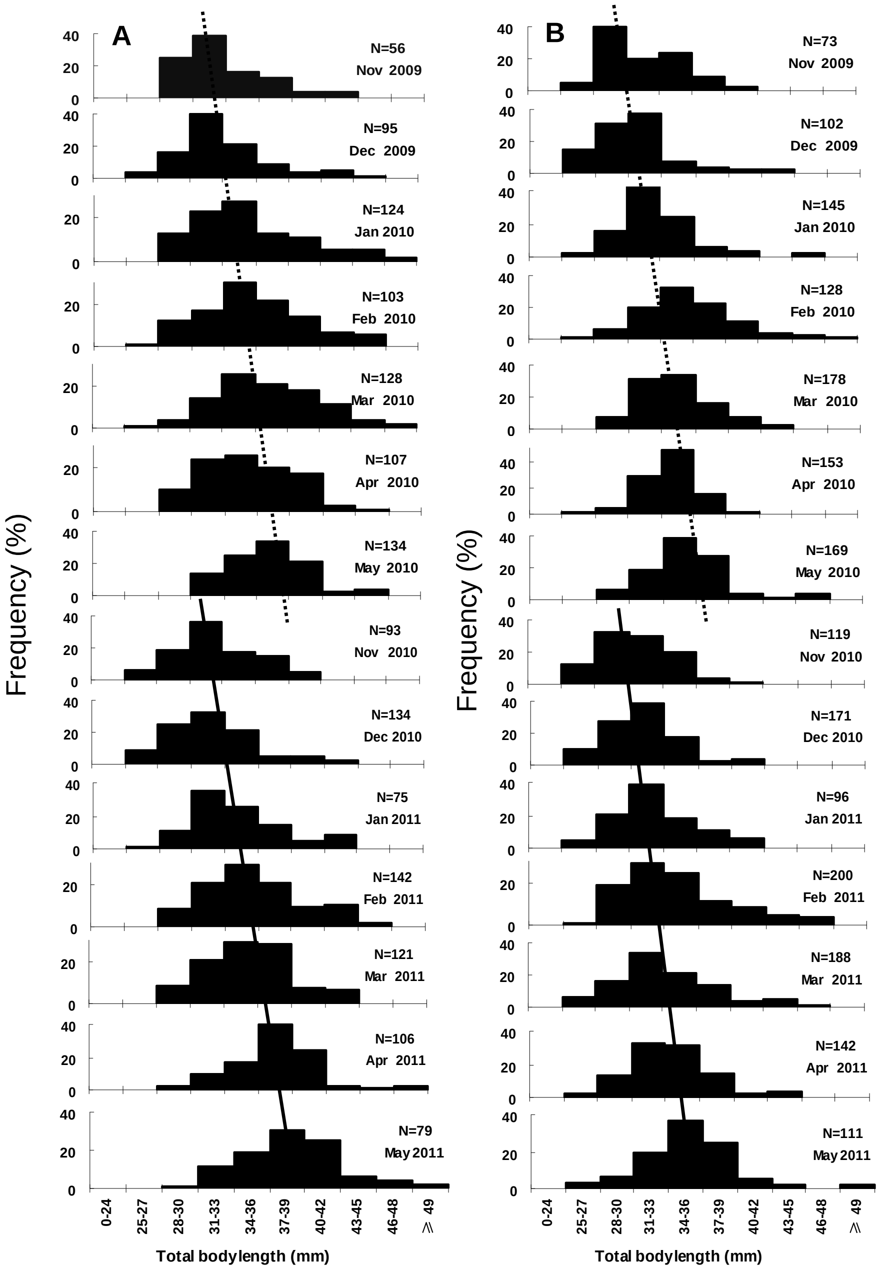

Fig. 2 Comparison of body length-frequency for (A) males and (B) females of *R. cliffordpopei* from November 2009 to May 2011 in Lake Erhai (China). The dotted line connects the cohort born in 2009. The solid line connects the cohort born in 2010.

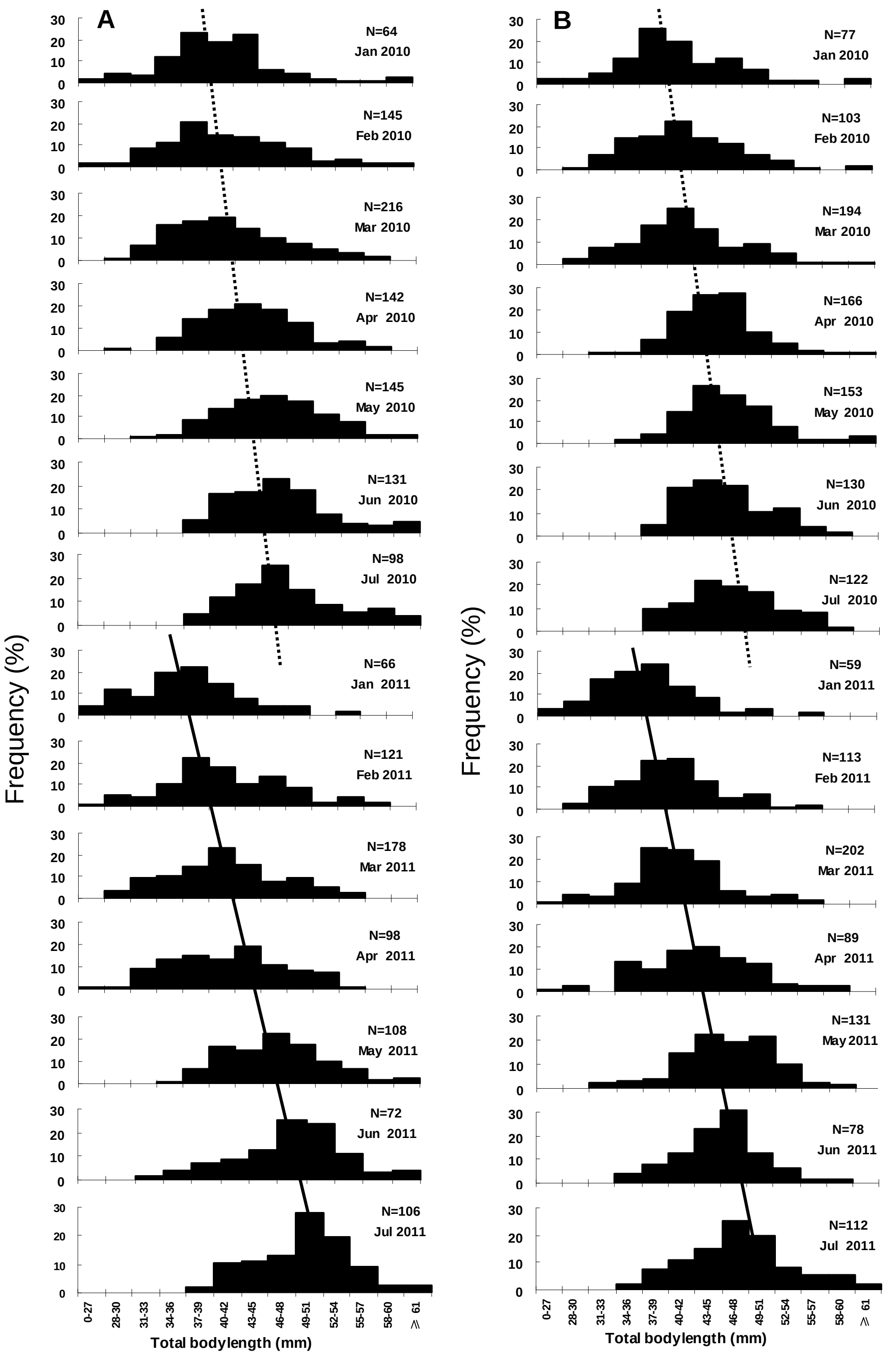

Fig. 3 Comparison of body length-frequency for (A) males and (B) femalesof *R. giurinus* from January 2010 to July 2011 in Lake Erhai (China). The dotted line connects the cohort born in 2009. The solid line connects the cohort born in 2010.

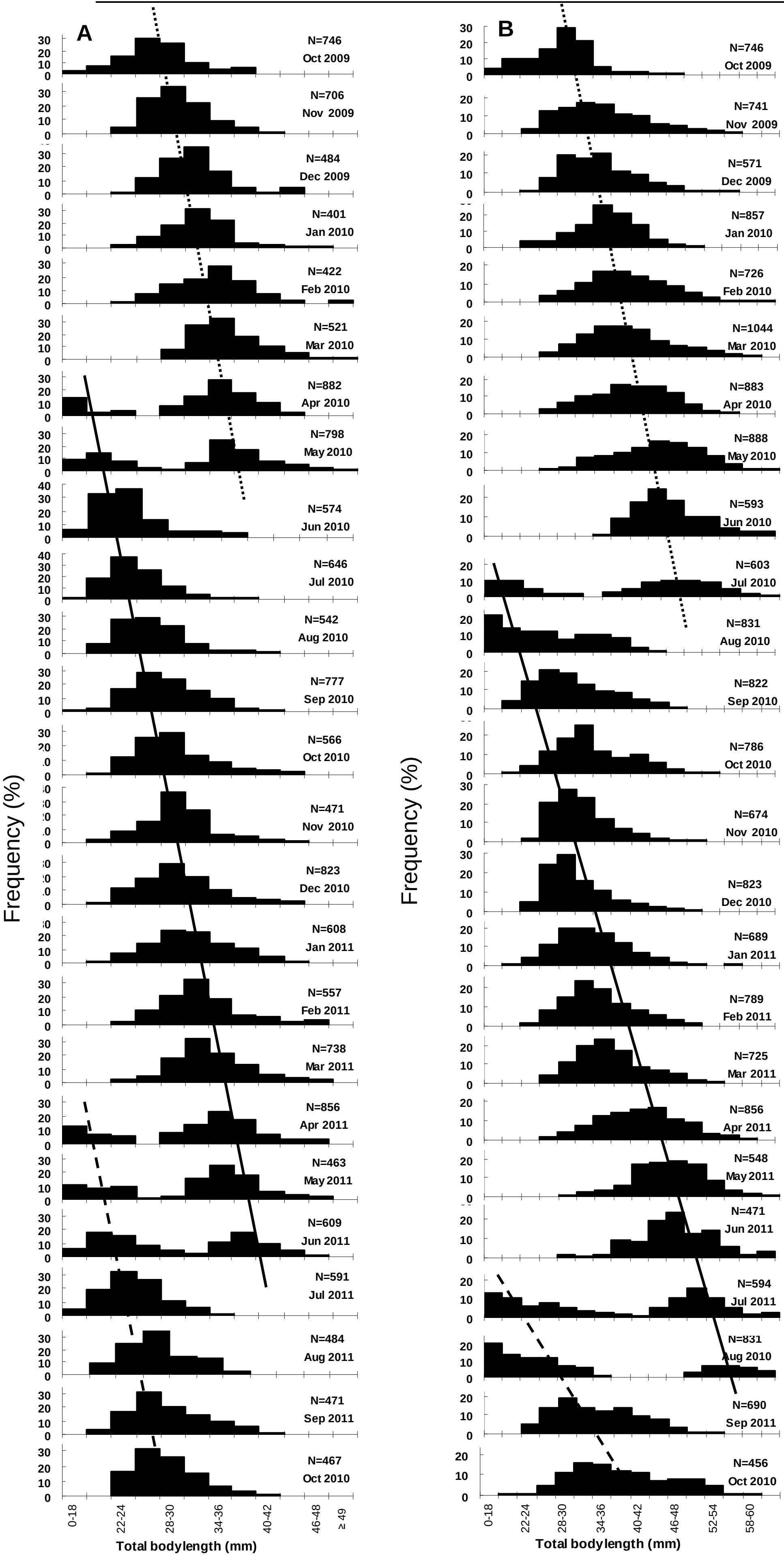

Fig. 4 Comparison of body length-frequency for the entire populations of (A) *R. cliffordpopei* and (B) *R. giurinus* from October 2009 to October 2011 in Lake Erhai (China). The dotted lines connect the cohort born in 2009. The solid lines connect the cohort born in 2010. The dashed lines connect the cohort born in 2011.

Table I Kolmogorov-Smirnov (K-S) test used to test differences in body length-frequency distributions of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		D	*P*	D	*P*	D	*P*
2009	Oct					0.149	0.126
	Nov	0.094	0.084			0.198	0.005
	Dec	0.311	<0.001			0.308	<0.001
2010	Jan	0.276	<0.001	0.095	0.779	0.181	<0.001
	Feb	0.138	0.153	0.079	0.836	0.098	0.009
	Mar	0.102	0.115	0.073	0.646	0.272	<0.001
	Apr	0.277	<0.001	0.158	0.043	0.533	<0.001
	May	0.071	0.212	0.115	0.275	0.614	<0.001
	Jun			0.103	0.486	0.949	<0.001
	Jul					0.534	<0.001
	Aug					0.363	<0.001
	Sep					0.183	<0.001
	Oct					0.373	<0.001
	Nov	0.276	<0.001			0.107	0.314
	Dec	0.091	0.172			0.098	0.896
2011	Jan	0.116	0.207	0.067	0.532	0.120	0.140
	Feb	0.097	0.140	0.101	0.579	0.057	0.409
	Mar	0.209	<0.001	0.117	0.604	0.114	0.143
	Apr	0.114	0.106	0.125	0.089	0.332	<0.001
	May	0.082	0.331	0.108	0.112	0.491	<0.001
	Jun			0.088	0.321	0.788	<0.001
	Jul					0.654	<0.001
	Aug					0.341	<0.001
	Sep					0.197	<0.001
	Oct					0.294	<0.001

Body length, body condition and growth parameters

In *R. cliffordpopei*, mean total body length (T_L) of males and females steadily increased from November to February but there was almost no further increase for females from March to May (Fig. 5). The males showed significantly higher T_L than females except in February, November and December 2010 (Mann-Whitney test, $P <$

0.05, Table II). Both sexes of *R. giurinus* showed a similar growth in T_L from January to July (Mann-Whitney test, P > 0.05, Table II & Fig. 5). For the entire populations, T_L of *R. cliffordpopei* peaked in March and dropped to the lowest values in July, while *R. giurinus* showed the highest $\mathrm{T_L}$ June and the lowest one in August (Fig. 5). Their T_L differed significantly in November 2009, January to July except March 2010, and April to October except August 2011 (Mann-Whitney test, P < 0.05, Table II).

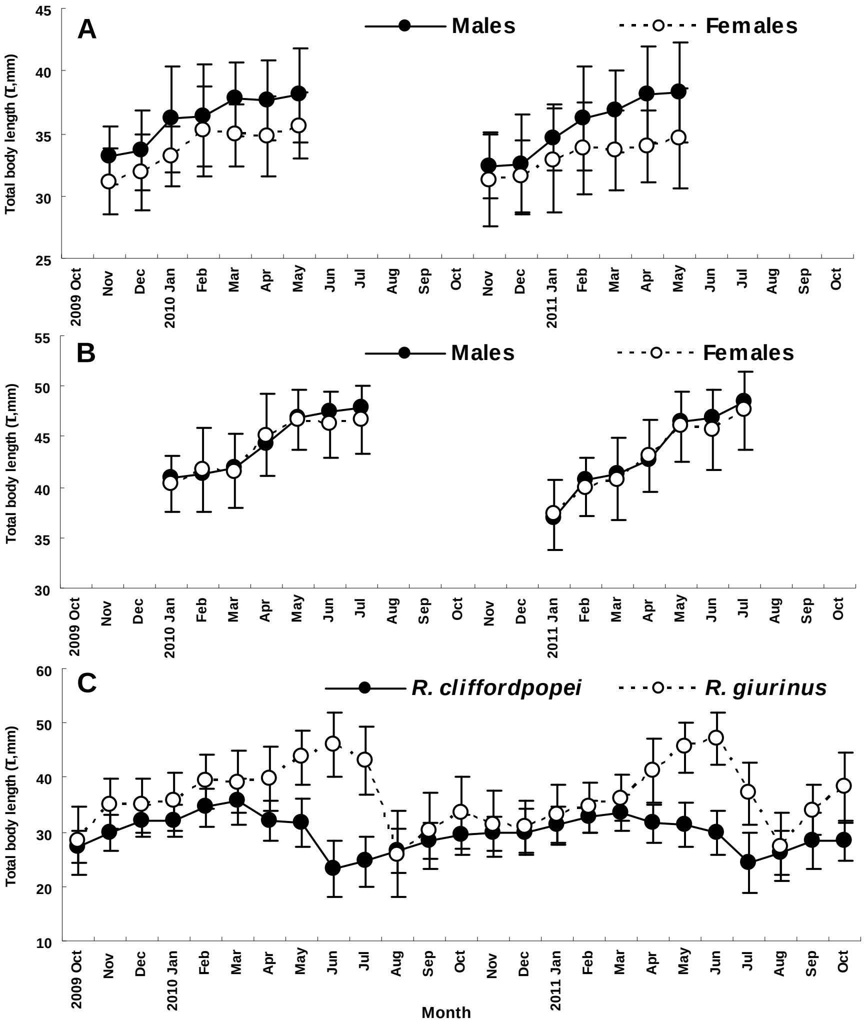

Fig. 5 Comparison of monthly changes in body length (mm) for males and females of (A) *R. cliffordpopei* and (B) *R. giurinus*, and (C) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (A) and (B) indicated that individuals unidentifiable for sexes.

Table II Mann-Whitney test used to test differences in total body length (mm) of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between	
		R.		*R. giurinus*		Entire	
		W	*P*	W	*P*	W	*P*
2009	Oct					14383	0.156
	Nov	29307	<0.001			71963	<0.001
	Dec	34881	<0.001			15435	0.089
2010	Jan	25143	<0.001	50941	0.687	14577	0.008
	Feb	10657	0.177	74114	0.847	19020	<0.001
	Mar	24571	0.001	21184	0.846	24578	0.104
	Apr	17451	<0.001	10543	0.110	65879	<0.001
	May	20167	<0.001	11659	0.446	54864	<0.001
	Jun			93141	0.189	12812	<0.001
	Jul					65815	<0.001
	Aug					79154	0.205
	Sep					30555	0.123
	Oct					45411	0.012
	Nov	14572	0.102			25827	0.156
	Dec	10294	0.114			37188	0.475
2011	Jan	24394	0.009	11274	0.501	27154	0.318
	Feb	24786	<0.001	20560	0.432	34182	0.167
	Mar	50149	<0.001	31476	0.389	17457	0.209
	Apr	43744	<0.001	28744	0.517	34715	<0.001
	May	54784	<0.001	76981	0.240	54724	<0.001
	Jun			34157	0.177	34781	<0.001
	Jul			14958	0.108	19785	<0.001
	Aug					45784	0.341
	Sep					24516	<0.001
	Oct					31254	<0.001

Body condition of females and males of *R. cliffordpopei* greatly increased in January and dropped in May (Fig. 6), whereas these of *R. giurinus* were highest in May and lowest in July (Fig. 6). Females showed significantly higher body condition than males from January to April for *R. cliffordpopei*, and from March to June for *R. cliffordpopei* (Mann-Whitney test, $P < 0.05$, Table III). The entire populations of *R. cliffordpopei* had higher body condition than *R. giurinus* in Oct 2009, January to

February to July 2010, and February to March 2011 (Mann-Whitney test, $P < 0.05$, Table III & Fig. 6).

Electronic Length Frequency Analysis (ELEFAN) demonstrated that the asymptotic length (L_∞) was 55.65 mm for *R. cliffordpopei* and 68.25 mm for *R. giurinus*. The rowth coefficient (K) was 0.58 for *R. cliffordpopei* and 0.99 mm for *R. giurinus.* The von Bertalanffy growth function by ELEFAN was $L_t = 55.65\,[- e^{-0.58\,(t + 1.48)}]$ (Score = 0.477) for *R. cliffordpopei* and $L_t = 68.25\,[- e^{-0.99\,(t + 0.78)}]$ (Score = 0.216, Fig. 7), respectively.

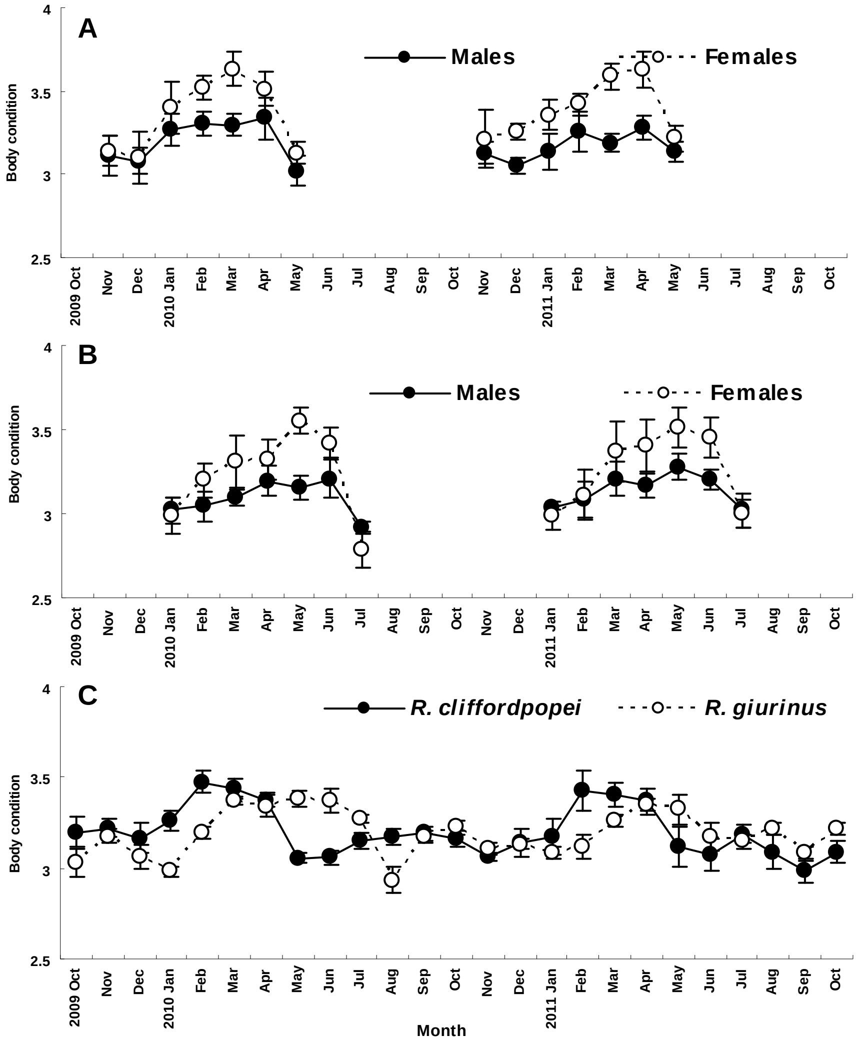

Fig. 6 Comparison of monthly changes in body condition females and females of (A) *R. cliffordpopei* and (B) *R. giurinus*, and (C) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (a) and (b) indicated that individuals unidentifiable for sexes.

Table III Analyses of covariance (ANCOVAs, total body length is a covariate) used to test differences in body condition of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011. The *F* and *P* values are effects of sexes on body condition within species, and effects of species on body condition for the entire populations.

		Between sexes within species				Between	
		R. cliffordpopei		*R. giurinus*		Entire	
		F	*P*	*F*	*P*	*F*	*P*
2009	Oct					16.771	<0.001
	Nov	0.907	0.343			1.797	0.184
	Dec	2.608	0.111			4.615	0.035
2010	Jan	3.140	0.089	2.2305	0.139	88.442	<0.001
	Feb	105.124	<0.001	10.104	0.002	53.264	<0.001
	Mar	122.830	<0.001	41.508	<0.001	2.3367	0.129
	Apr	6.118	0.016	20.342	<0.001	0.868	0.354
	May	6.515	0.012	89.543	<0.001	93.635	<0.001
	Jun			31.875	<0.001	77.237	<0.001
	Jul			11.678	0.001	7.569	0.007
	Aug					37.455	<0.001
	Sep					0.382	0.5381
	Oct					2.981	0.087
	Nov	3.461	0.066			1.379	0.243
	Dec	24.563	<0.001			1.936	0.167
2011	Jan	19.876	<0.001	2.804	0.098	0.531	0.467
	Feb	9.211	0.003	1.871	0.175	50.212	<0.001
	Mar	18.982	<0.001	44.630	<0.001	7.390	0.008
	Apr	145.622	<0.001	79.381	<0.001	0.352	0.555
	May	1.954	0.161	27.564	<0.001	24.213	<0.001
	Jun			34.070	<0.001	7.092	0.009
	Jul			2.329	0.131	3.558	0.063
	Aug					4.759	0.0319
	Sep					2.878	0.093
	Oct					6.021	0.016

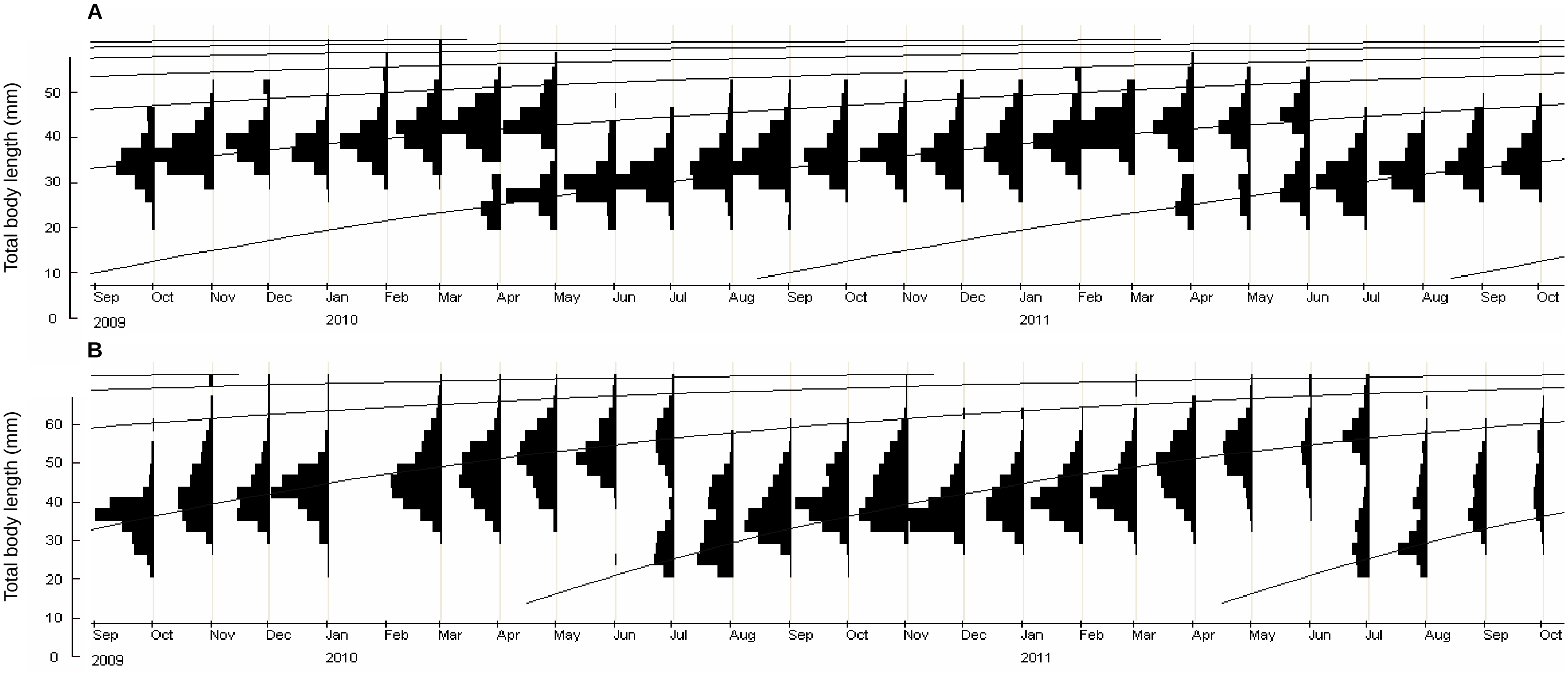

Fig. 7 Growth lines of (A) *R. cliffordpopei* and (B) *R. giurinus* obtained by Electronic Length Frequency Analysis (ELEFAN). The von Bertalanffy growth function by ELEFAN was $L_t = 55.65\,[- e^{-0.58\,(t\,+\,1.48)}]$ (Score = 0.477) for *R. cliffordpopei* and $L_t = 68.25\,[- e^{-0.99\,(t\,+\,0.78)}]$ (Score = 0.216), respectively.

Mortality, longevity and abundance dynamics

R. cliffordpopei showed higher total mortality ($Z = 2.12$), fishing mortality ($F = 1.25$), exploitation rate ($E = 0.59$) than that of *R. giurinus* ($Z = 1.73$, $F = 0.56$, $E = 0.32$), whereas the natural mortality ($M = 0.85$) of *R. cliffordpopei* was lower than of *R. giurinus* ($M = 1.17$, Fig. 8).

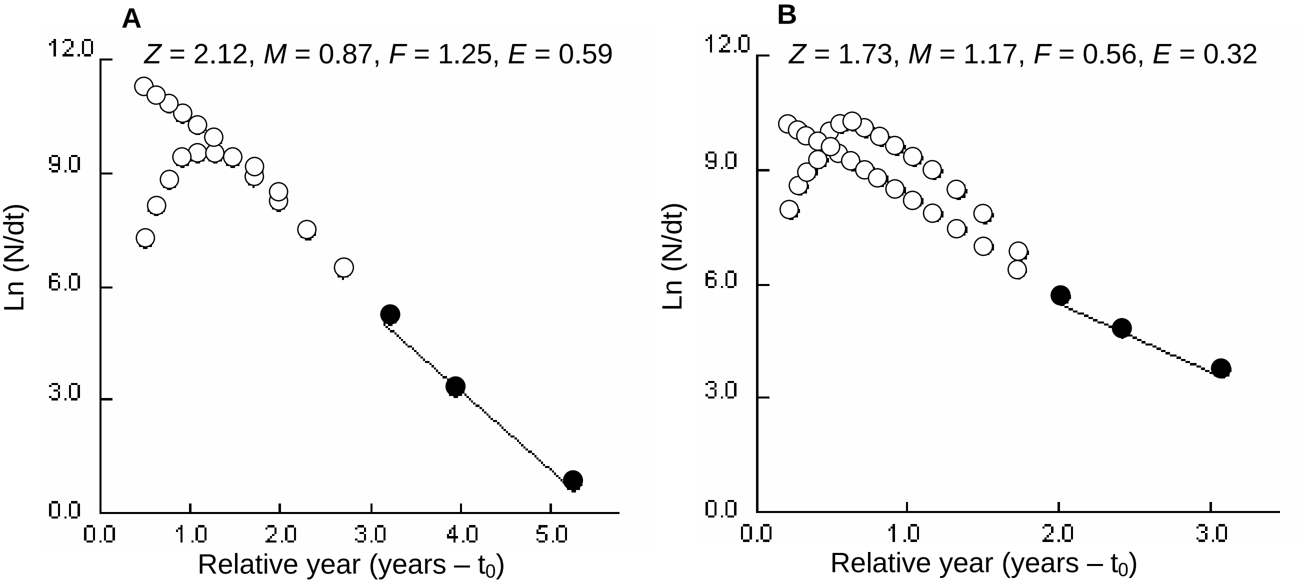

Fig. 8 Body length converted catch curve and mortality estimates for (A) *R. cliffordpopei* and (B) *R. giurinus* in Lake Erhai (China) from October 2009 to October 2011. The estimations were conducted using the parameters of the von Bertalanffy growth function and mean temperature of 18.7°C. *Z*, total mortality; *M*, natural mortality; *F*, fishing mortality; *E*, exploitation rate. The regression was fitted using ● data points on the curve.

The mean size at first sexual maturity (L_{50}) and mean time at first sexual maturity (T_{50}) was 35.4 mm and 0.33 years for *R. cliffordpopei*, which is lower than 46.9 mm and 0.39 years for *R. giurinus* (Fig. 9). The maximum longevity (T_m) was 1.23 and 1.45 years for *R. cliffordpopei* and *R. giurinus*, respectively.

The catch per unit effort (*CPUE*) differed significantly between sexes in *R. cliffordpopei* (RM ANOVAs, $F = 55.372$, d.f. = 1, $P < 0.001$, Table IV) but insignificantly in *R. giurinus* (RM ANOVAs, $F = 2.508$, d.f. = 1, $P = 0.122$, Table IV). The females of *R. cliffordpopei* showed significantly higher *CPUE* than males in November 2009, February to May 2010 and 2011 (Mann-Whitney test, $P < 0.05$, Table V & Fig 10).

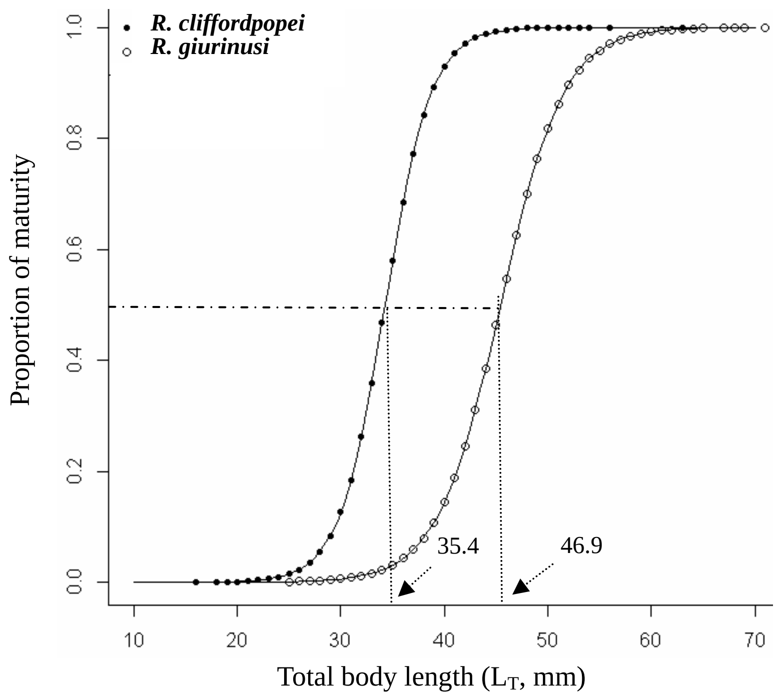

Fig. 9 Comparison of the size of maturity of *R. cliffordpopei* (n = 4112) and *R. giurinus* (n = 4541s) from October 2009 to October 2011 in Lake Erhai (China). Mean size of maturity (L_{m50}) was 35.4 mm and 46.9 mm for *R. cliffordpopei* and *R. giurinus*, respectively.

For the entire populations, the *CPUE* were significantly different between species (RM ANOVAs, F = 74.549, d.f. = 1, $P < 0.001$, Table IV). *R. cliffordpopei* displayed higher *CPUE* than *R. giurinus* in July to August 2010, July to September 2011, whereas *R. giurinus* showed higher *CPUE* than *R. cliffordpopei* in April to June and October to December 2010, and April to June 2011 (Mann-Whitney test, $P < 0.05$, Table V & Fig. 10). Moreover, months had significantly effect on *CPUE* of males and females (RM ANOVAs, F = 52.106, d.f. = 3.411, $P < 0.001$ for *R. cliffordpopei* and F = 109.653, d.f. =3.549, $P < 0.001$ for *R. giurinu*, Table IV), and entire populations of the two gobies (F = 29.892, d.f. = 6.445, $P < 0.001$, Table IV). The males, females and entire population showed the lowest *CPUE* from December 2009 to February 2010 and December 2010 to February 2011, during which the water temperature was also lowest (Fig. 10). The *CPUE* of entire populations was low from April to June in *R. cliffordpopei* and from July to September in *R. giurinus,* whereas it was high from July to September in *R. cliffordpopei* and from April to June, September to November in *R. giurinus* (Fig. 10).

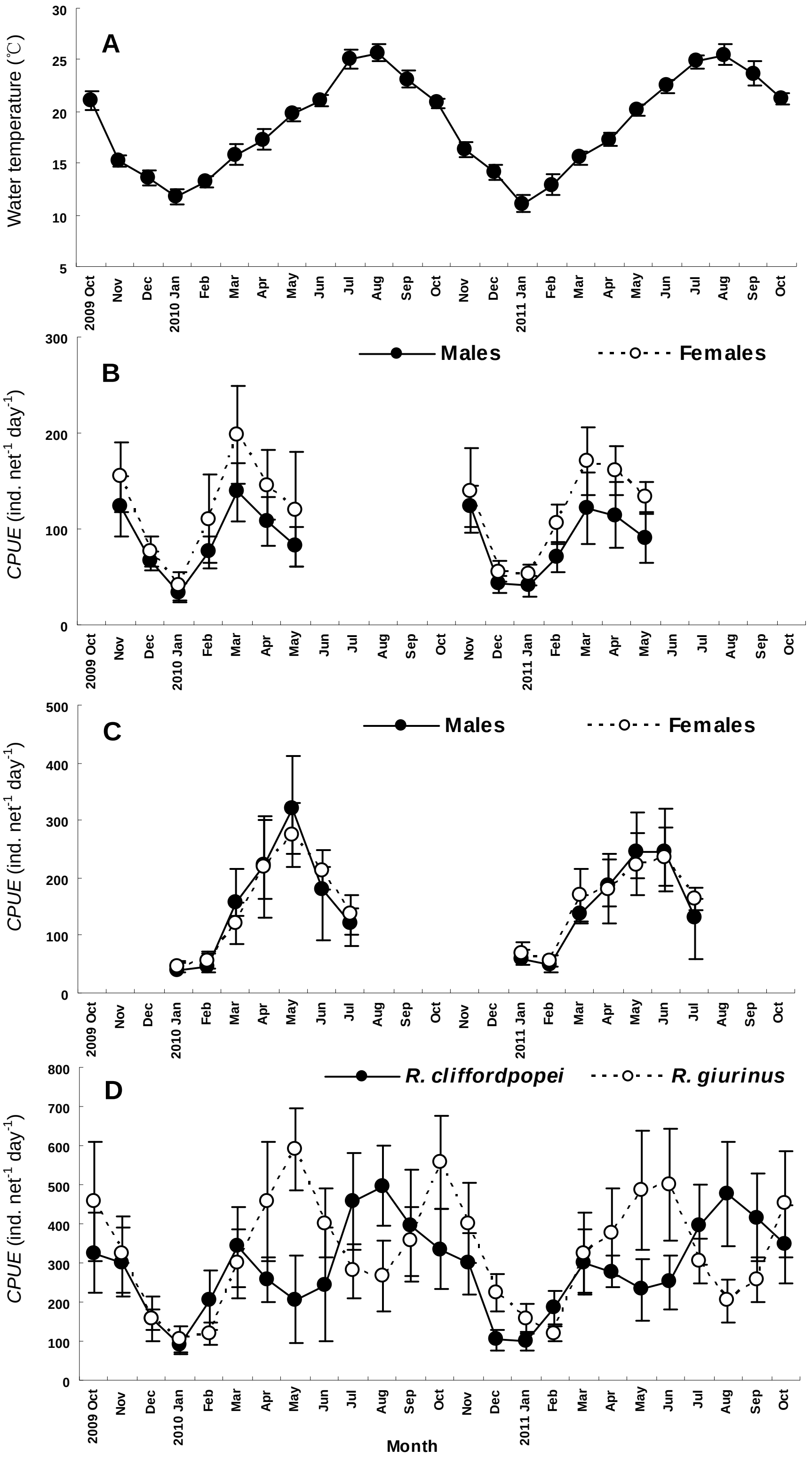

Fig. 10 Monthly changes in (A) water temperature and comparison of catch per unit effort (*CPUE*) for males and females of (B) *R. cliffordpopei* and (C) *R. giurinus*, and (D) their entire populations in Lake Erhai (China) from October 2009 to October 2011. The absence of data in (B) and (C) indicated that individuals unidentifiable for sexes.

Table IV Repeated-measures ANOVAs (RM-ANOVA) used to test differences in cath per unit efforts (CPUE, log-transformed) of *R. giurinus* and *R. cliffordpopei* between sexes (fixed factor) within species, and between species (fixed factor) for the entire populations with months as random factors in Lake Erhai (China) from October 2009 to October 2011.

	Sources	SS	d. f.	MS	*F*	*P*
R. cliffordpopei	Sexes	3.658	1	3.658	55.372	<0.001
	Error	1.454	22	0.066		
	Months	50.175	3.411	14.710	52.106	<0.001
	Error	21.184	75.040	0.028		
	Sexes × Months	5.282	3.549	1.488	3.406	0.014
R. giurinus	Sexes	0.267	1	0.267	2.508	0.122
	Error	4.043	38	0.106		
	Months	170.028	3.549	47.913	109.653	<0.001
	Error	58.923	134.849	0.437		
	Sexes × Months	2.190	3.411	0.642	2.274	0.079
The entire populations	Species	5.800	1	5.800	74.549	<0.001
	Error	2.334	30	0.078		
	Months	75.730	6.445	11.750	29.892	<0.001
	Error	76.004	193.352	0.393		
	Species × Months	83.056	8.671	9.579	32.784	<0.001

Table V Mann-Whitney test used to test differences in catch per unit effort (*CPUE*) of *R. cliffordpopei* and *R. giurinus* between sexes within species, and between species for the entire populations in Lake Erhai (China) from October 2009 to October 2011.

		Between sexes within species				Between species	
		R. cliffordpopei		*R. giurinus*		Entire populations	
		W	*P*	W	*P*	W	*P*
2009	Oct					240	<0.001
	Nov	21	0.003			89	0.239
	Dec	47	0.148			128	0.754
2010	Jan	57	0.399	172	0.456	147	0.293
	Feb	18	0.002	155	0.2267	41	0.002
	Mar	9	<0.001	131	0.073	112	0.105
	Apr	15	0.001	184	0.533	44	0.003
	May	27	0.009	117	0.026	215	<0.001
	Jun			135	0.081	254	<0.001
	Jul			152	0.199	233	<0.001
	Aug					200	<0.001
	Sep					109	0.097
	Oct	160	0.288			47	<0.001
	Nov	40	0.071			214	<0.001
	Dec	45	0.105			35	<0.001
2011	Jan	51	0.218	188	0.755	202	<0.001
	Feb	18	0.002	192	0.839	191	0.005
	Mar	12	<0.001	139	0.089	100	0.459
	Apr	30	0.009	144	0.130	128	0.007
	May	15	0.001	162	0.293	228	<0.001
	Jun			149	0.170	178	0.002
	Jul			151	0.101	33	<0.001
	Aug					56	<0.001
	Sep					14	<0.001
	Oct					99	<0.001

Discussion

The life span of gobies varies greatly with species (Azevedo & Simas, 2000; Scalici & Gibertini, 2009; Shemonaev & Kirilenko, 2009) and several of them show short-lived life cycle (e.g. about one year for *Eucyclogius newberryi* (Girard, 1856) (Swcnson, 1999) and *Istigobius decoratus* (Herre, 1927) (Kritzer, 2002)). In Lake Erhai, the age of the two gobies species is 0+ determined by scales, which is consistent with the findings in native lakes along the middle and lower reaches of the Yangtze River (Zhang, 2005). Moreover, the body length-frequency was unimodal in most cases for the two gobies and changed suddenly when the new born individuals grew large enough to be caught. The large adults decreased and/or disappeared after two or three months of spawning. This clearly demonstrated that adults probably died after spawning seasons and the life span of the two species is about one year. The maximum longevity estimation is convinced us of this results (i.e. 1.23 for *R. cliffordpopei* and 1.45 years for *R. giurinus*).

Moreover, the cohort structures of the two species revealed that new born individuals appeared at different months by benthic fyke nets (i.e. new born cohort appeared in April for *R. cliffordpopei* and in July for *R. giurinus*). Since *R. cliffordpopei* spawned from February to June with the peaking season in March to April, and *R. giurinus* spawned from April to August with the peaking season in May to June (Guo *et al*., 2012b), the different time for new born cohort closely match spawning seasons of the two species. Those differences are crucial for the mesh size of benthic fyke nets in physical removal, the timing of deployment of remediation actions as well as the controlling methods (Knapp & Matthews, 1998; Ling, 2002; Britton *et al*., 2011). Specifically, in order to remove the small-sized individuals, the mesh sizes of benthic fyke nets should be less than 4 mm from March to June for *R. cliffordpopei* and from June to August for *R. giurinus* because nets with mesh sizes of 4 mm were inefficient for those individuals. Moreover, as offsprings of fishes usually increase several orders of magnitude population size and disperse intensively, management operations often most economic and effective when implemented pre-spawning and some studies even demonstrated the removals of adults soon after spawning could result in a population increase in juveniles (e.g. decreased intraspecific competition) rather than their decline (Ludgate & Closs, 2003; Wimbush *et al*., 2009). Thus, based on the characteristics of cohort structures and spawning seasons, the eradication actions or physical removal are highly recommended to be strengthened from September to February for *R. cliffordpopei*, and from February to April for *R. giurinus* with the aim at decreasing the mature populations (Guo *et al*., 2012b). In addition, most small-sized individuals (i.e. fish larvae) are pelagic from February to June for *R. cliffordpopei* and from April to August for *R. giurinus*, the benthic fyke nets are useless before those fish larvae grow big enough to settle into bottom. In these periods, other controlling methods are very likely to be more efficient than benthic fyke nets, such as chemical treatments (e.g. rotenone, Ling, 2000), electricity fishing (Britton *et al*., 2011), and/or light trapping (Meekan *et al*., 2001; Vilizzi *et al*., 2008).

Although several models have been proposed to estimate the growth of fishes (e.g. Gompertz model (Gompertz, 1825), logistic model (Ricker, 1975)), the most widely used one is the von Bertalanffy growth model (von Bertalanffy, 1938) because its parameters are particularly useful in describing fish growth, estimating life parameters and deriving fisheries reference points (Cope & Punt, 2007; Katsanevakis & Maravelias, 2008; Helidoniotis *et al*., 2011). However, the growth patterns of fishes typically analyze age-structured demography in which the short-lived species are currently often overlooked (Taylor *et al*., 2005). A few studies have estimated the growth parameters of freshwater gobies (e.g. Azevedo & Simas, 2000; Scalici & Gibertini, 2009) but, to our knowledge, such information is extremely scarce for species of the genus of *Rhinogobius.* In present study, von Bertalanffy growth parameters of the two gobies were firstly estimated. The growth rate of those two gobies (0.58 year^{-1} for *R. cliffordpopei* and 0.99 year^{-1} for *R. giurinus*) seems to not vary greatly with the values for other gobies such as 0.89 or 0.76 year^{-1} for rock goby *Gobius paganellus* (Linnaeus, 1758) in Ponta Delgada of Azores (Azevedo & Simas, 2000) and 0.56 – 0.68 year^{-1} for Italian goby *Gobius nigricans* (Canestrini, 1867) in River Farfa of Italy (Scalici & Gibertini, 2009). In Lake Erhai, *R. giurinus* grows faster than *R. cliffordpopei*. This generally agreed with the monthly changes in body length whereby *R. giurinus* showed larger body size and also a faster increase than *R. cliffordpopei.* However, the fluctuation of mean body length of the two species were apparently different (*R. cliffordpopei* showed the highest values in March and lowest June or July, while *R. giurinus* showed the lowest values in June and lowest in August). Combined the patterns of cohort structures, monthly changes of mean body length is the most important criterion for determining the mesh size of benthic fyke nets used for physical removals.

Here, significant variations of *CPUE* of those two speceis among different months were mainly due to the changes in activity level and recruitment cycles (Xie *et al.* 2000; Guo *et al*., 2012b). In winter, the *CPUE* of two species was extremely low, which showed a considerable discrepancy with the findings in Biandantang Lake (a shallow lake along the middle and lower reach of the Yangtze River), where the individuals of *R. giurinus* caught were 343 in winter and that was slightly lower than 412, 436 and 347 in spring, summer and autumn (Xie *et al*., 2000). Benthic trap nets, like most of passive nets system, are highly dependent on activity level of fish and it quite differed with quantitative sampling method by pop nets in Biandantang Lake (Xie *et al*., 2000). Thus, the significantly lower *CPUE* of two species in winter mainly resulted from their lower activity level when the water temperature was low rather than recruitment patterns (e.g. adults dying after spwaning). However, the *CPUE* showed significantly low values in April to June for *R. cliffordpopei* and in July to September for *R. giurinu.* This strongly coincided with death of adults after spawning. After those periods, the *CPUE* increased clearly resulting from the newly settled juveniles.

The seasonal changes in *CPUE* of the benthic fyke nets should be incorporated into the management strategies from the perspective of low costs-efficiency since the mainly controlling actions in the lake are currently the physical removal by fyke nets. For *R. cliffordpopei*, the physical removal in winter is recommended to be redoubled despite the significantly lower *CPUE* because the actions during this period aim at decreasing the mature populations as stated above. Moreover, because *R. cliffordpopei* mostly uses the littoral zones of the lake, the low abundance of submerged macrophytes in winter can facilitates an easy setting and retrieving of nets. For *R. giurinu*, however, the physical removal is suggested to be lessened or concealed during winter as such operations in profundal zones (water depths range 12 – 20 m) are energy- and time-costly. In the other low *CPUE* periods (i.e. from April to June for *R. cliffordpopei* and from July to September for *R. giurinu*), the physical removal using fyke nets probably can be lessen and/or transferred to other outlets (e.g. electricity fishing, light trap, etc.). In contrary, the removal actions should be strengthened when the *CPUE* are high July to September for *R. cliffordpopei* and from April to June, September to November in *R. giurinus.*

In conclusion, the present study demonstrated that those two goby species are short-lived with a life span of one year. The growth patterns and population dynamics are different between the two species. Based on such findings, specific strategies are proposed to improve the current management strategy and develop the possible new management program for the two pest species in the lake. However, since the current control operations taken in Lake Erhai are almost physical removal by fyke nets, the synergistic remediation are highly recommend as a complementary strategy such as chemical treatments, electricity fishing removals, light trapping and/or bio-controlling (Ling, 2000; Britton *et al*., 2011). Studies are needed to quantify the efficiency of these synergistic programs and evaluate their potential effects on non-target species and introduced ecosystem, especially for chemical treatments and bi-controlling.

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Comparative study of the reproductive biology of two congeneric and introduced goby species: implications for management strategies

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Abstract

A full understanding of life characteristics of invasive species is a fundamental prerequisite for the development of management strategies. Two introduced goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) have established highly abundant populations in Lake Erhai (China). In the present study, we examined the reproductive biology of these two species with the aim of facilitating a more effective management strategy. The results indicated that *R. cliffordpopei* spawned from February to June, whereas *R. giurinus* spawned from April to August. *R. cliffordpopei* showed higher gonado-somatic indices and had larger eggs over *R. giurinus*. The adult sex ratio of *R. cliffordpopei* was female-skewed but that of *R. giurinus* was equal. *R. cliffordpopei* showed a male-skewed sexual size dimorphism whilst the body size of *R. giurinus* varied only slightly between males and females. The different reproductive traits appear as a crucial biological aspect for developing control programs. Specifically, control measures should be implemented and/or intensified from September to February for *R. cliffordpopei,* and from January to April for *R. giurinus*. The body size of the smaller *R. cliffordpopei* females is the determinant for minimal mesh size of the nets used in physical removals of *R. cliffordpopei*.

Key words: goby, biological invasion, reproductive strategy, invasive species management

Introduction

As more and more introduced species are establishing invasive populations worldwide and impacting recipient ecosystems (Karatayev et al., 2009; Cucherousset & Olden, 2011; Winfield et al., 2011), increasing attention has been devoted to their management during the last decade (Sutherland et al., 2009; Gozlan et al., 2010). Specifically, several measures (e.g. physical removal, chemical eradication and bio-manipulation) have been developed and implemented to control, contain or eradicate a wide range of invasive species (Taylor & Hastings, 2004; Saunders et al., 2010; Britton et al., 2011). The effectiveness of these measures is, however, highly dependent upon the life characteristics of the target invasive species (Ludgate & Closs, 2003; Taylor & Hastings, 2004; Britton et al., 2011; Yeates et al., 2012). For instance, the eggs of fish species such as salmonids are around 100 times less sensitive to rotenone than juveniles and adults (Marking & Bills, 1976), and therefore eradications using rotenone should account for different vulnerabilities among life stages (Ling, 2002; Britton et al., 2011). Consequently, a full understanding of life characteristics of invasive species is crucial to develop economically and/or ecologically effective management strategies (Ludgate & Closs, 2003; Taylor & Hastings, 2004; Yeates et al., 2012). Reproduction characteristics can have substantial effects on invasion success, and a thorough knowledge of the reproduction biology of invasive species is a fundamental prerequisite for developing appropriate management strategies.

Many goby species have been introduced worldwide and have become established outside of their native ranges, including in North American (Dillon & Stepien, 2001; Copp et al., 2005), Europe (Copp et al., 2005; Antsulevich, 2007) and Asia (Du et al., 2001; Yuan et al., 2010). In the Great Lakes, for instance, several goby species native from the Ponto-Caspian area (e.g. round goby *Apollonia melanostoma*, tubenose goby *Proterorhinus semilunaris*, racer goby *Neogobius gymnotrachelus*) have spread in the entire system (Dillon & Stepien, 2001; Kolar & Lodge, 2002). In Europe, several goby species (e.g. round goby, bighead goby *Neogobius kessleri*, monkey goby *Neogobius fluviatilis* and racer goby) have also invaded new ecosystems (Copp et al., 2005). In China, introduced gobies of the genus *Rhinogobius* have established in most lakes of Yunnan-Guizhou Plateau (Xie et al., 2001, Yuan et al., 2010). The rapid proliferation of these species has raised serious concerns regarding their long-term negative impacts on native species and ecosystems (Xie et al., 2001; Cooper et al., 2007; Krakowiak & Pennuto, 2008; Yuan et al., 2010), but few studies have paid attention to the development of specific management strategies for these invasive goby species.

Rhinogobius cliffordpopei and *Rhinogobius giurinus* are two common small-bodied goby species from central and eastern China. They were inadvertently introduced into most lakes of the Yunnan Province in the 1950-60s and their populations have expended greatly over the last decades (Xie et al., 2001; Yuan et al., 2010). The two species were introduced simultaneously to Lake Erhai in 1961 and since then have become the most dominant benthic fish species (Du & Li, 2001). The annual yield of the two species accounts for 48 % of total fish yields (kg) of the lake in 2010. The two species mainly prey on large-sized zooplanktons (Cladocera and Copepoda) and aquatic insects (Chironomid larvae), and they often prey on fish larvae and eggs, including those from native species (Xie et al., 2000; Du et al., 2001; Zhang, 2005). When the populations are highly abundant, they can compete with native species (e.g. *Cyprinus longipectoralis, Cyprinus barbatus, Barbodes daliensis*) for food resources (Du et al., 2001). The two species are considered to be one of the major causes of the decline and/or extirpation of some native fishes in the lake. In the native lake along the middle and lower reaches of the Yangtze River (China), the two species have been described as ecologically similar with close life history characteristics and feeding habits (Zhang 2005; Wu & Zhong, 2008). For instance, Zhang (2005) have studied the reproductive biology of the two species in a native lake while Yan & Chen (2007) have compared spawning seasons and female fecundity of *R. giurinus* in two native lakes and an introduced lake. However, no study has compared the reproductive biology of the two species outside of their native range with the aim of developing management strategies.

Currently, the principal management strategy to control these two invasive species in Lake Erhai is similar (i.e. removals by the local fishery) despite the fact that they might differ in many aspects of their life history, including their reproductive biology. Therefore, quantifying the reproductive biology of the two species outside of their native range may promote the development of a more effective management strategy. Here, the reproductive biology of the two goby species was compared to test whether the species differ regarding seasonal spawning cycles, trade-offs between egg size and fecundity, adult sex ratio, size at maturity and sexual size dimorphisms (SSD). Such a comparison is expected to facilitate a species-specific management strategy for the two invasive species.

Materials and Methods

Study area

The present study was conducted in Lake Erhai (105°5-17′ E, 23°35-58′ N), a shallow freshwater plateau lake in northwest of the Yunnan Province in China (Fig. 1). Water temperature usually peaks at around 25-27 °C in July or August without thermal or dissolved oxygen stratification and drops to approximately 6-8 °C in December or January. The dominant fish species of Lake Erhai are small-bodied, primarily non-native species. Previous investigations have demonstrated that two species displayed strong habitat segregation whereby *R. cliffordpopei* mainly uses littoral habitat (LH, water depth < 6 m, high abundance of submerged macrophytes, submersed-macrophyte detritus substrata) and *R. giurinus* mainly uses profundal habitat (PH, 12 m < water depth < 12 to 20 m, no submerged macrophytes, silt and coarse sand substrata, Table 1).

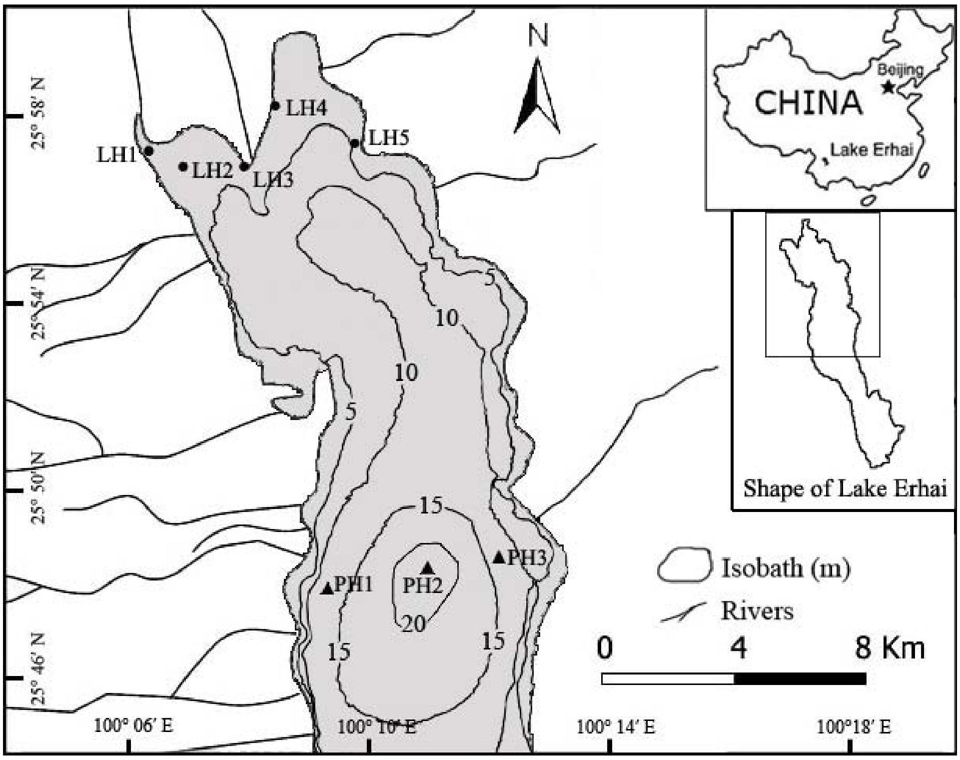

Fig. 1 Sampling sites of *R. cliffordpopei* in littoral habitat (●, LH1-LH5) and *R. giurinus* in profundal habitat (▲, PH1-PH3) in the central and northern parts of Lake Erhai (China).

Table l Environmental characteristics and abundances of two goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) in littoral habitat (LH) and profundal habitat (PH) in Lake Erhai (China). Parameters were measured in 2010 and reported values are mean ± standard deviation ("n" is the number of replication).

Environmental variables	Littoral habitat (LH)	Profundal habitat (PH)
Water depth (m)	3.1± 1.2 (n=40)	15.9 ± 3.1 (n=24)
Water temperature (°C)	16.9 ± 4.7 (n=40)	16.9 ± 4.6 (n=24)
Submerged macrophytes (g m^{-2})	3307 ± 881 (n=40)	0 (n=24)
pH	9.33 ± 0.32 (n=40)	9.08 ± 0.26 (n=24)
Secchi depth (cm)	214 ± 36 (n=40)	255 ± 25 (n=24)
Conductivity (μScm^{-1})	237 ± 17 (n=40)	234 ± 13 (n=24)
Dissolved oxygen (mg L^{-1})	9.23 ± 1.56 (n=40)	7.51 ± 0.88 (n=24)
Total nitrogen (mg L^{-1})	0.46 ± 0.12 (n=20)	0.43 ± 0.05 (n=12)
Total phosphorus (mg L^{-1})	0.0138 ± 0.0081 (n=20)	0.0142 ± 0.0069 (n=12)
Chlorophyll *a* (mg L^{-1})	8.27 ± 2.88 (n=20)	12.44 ± 3.09 (n=12)
R. cliffordpopei (ind. net^{-1} day^{-1}) [1]	250 ± 156 (n=160)	37 ± 21 (n=96)
R. giurinus (ind. net^{-1} day^{-1}) [1]	59 ± 28 (n=160)	318 ± 268 (n=96)

[1] Mean number of each species collected per benthic fyke net for twenty-four hours

Fish sampling and data collection

From October 2009 to October 2011, *R. cliffordpopei* and *R. giurinus* were sampled monthly using benthic fyke nets in five sites in LH (LH1-LH5) and three sites in PH (PH1-PH3), respectively (Fig. 1). The nets comprised a trunk stem with twenty traps, two end-traps and two end-pockets. Total length of the net was 15 m, including 12 m of traps (0.6 m each), 2 m of end traps (1 m each) and 1 m of end pockets (0.5 m each). The framework of each trap was made of iron wire with a width of 0.35 m and a height of 0.62 m. The stretched mesh size was 4mm. Four nets were deployed per site and per month from 8:00 to 12:00. A stone was added in each end of the nets and two ropes with buoys were attached to each end pocket of the nets (the ropes were longer than 15m in PH). After 24 hours, the nets were retrieved by pulling slowly the ropes and all catches in the end pockets were collected. The sampling procedure was similar in the LH and PH. All *R. cliffordpopei* collected from five sampling sites in the littoral habitat were pooled in each month. A random sub-sample was subsequently taken. The same procedure was used for *R. giurinus* in the profundal habitat. For each month and each species, the number of individuals subsequently analyzed ranged from 200 to 300. The sub-samples were kept at -20 °C in a refrigerator for subsequent analyses. Total body length (L_T) and body mass (M_T) were measured for each individual to the nearest mm and 0.01 g. Based on macroscopic appearances, gonads of both sexes were classified into five reproductive stages (stage I to V) following Yan & Chen (2007) (Table 2). Developmental stages of ovaries and testes were assessed by visual inspection primarily based on its appearances (Table 2). Gonads (≥ stage II, Table 2) were carefully removed, weighed to the nearest 0.l mg (gonad mass, M_G). The sex (female or male) of each individual was determined by macroscopic differences of ovaries and testes (≥ stage II, Table 2). Eviscerated mass (M_E) of each individual was measured. To estimate female fecundity, about 0.1 g of mature ovaries (stage IV at the peak of the breeding season, Table 2) were sampled from the anterior, middle and posterior sections of each lobe. The samples were weighed (M_S) and fixed with 10 % formalin solution for two weeks and then preserved in 75 % ethanol. The number of eggs in each sub-sample (N_S) was subsequently determined and the length and width of the eggs measured under an optical microscope. Diameter of each egg (D_E) was calculated based on the mean values of length and width. All the procedures were performed following the legislation in China.

Table 2 Macroscopic characteristics (modified from Yan & Chen (2007)) used to classify gonad maturity stages of *R. cliffordpopei* and *R. giurinus* in Lake Erhai (China)

Gonad maturity stages	Female	Male
I (virgin stage)	Sexes are indistinguishable by naked eye; gonads are thin, threadlike and transparent	Sexes are indistinguishable by naked eye; gonads are thin, threadlike and transparent
II (immature stage)	Ovaries are small, translucent or pale-yellow, more rod-shaped than stage-I; oocytes are indiscernible	Testes are lender, white or gray and larger than stage-I s
III (maturing stage)	Ovaries are swelling, orange and occupy 1/5 to 1/3 of body cavity; vitellogenic oocytes are tightly packed in ovaries and can be visible from epithelium	Testes are firm, flat-shaped and ivory white
IV (spawning stwage)	Ovaries occupy 1/3 to 2/3 of body cavity; oocytes attain their maximum volume and can be released from genital pore with light abdominal pressure	Testes are ivory white and milt can be released genital pore with light pressure on the abdomen
V (spent stage)	Ovaries are flaccid and sometimes red with visible capillaries; there are often a few of residual oocytes in ovaries	Testes are flaccid and deceased volume clearly; milt are found in some individuals

Statistical analyses

The gonado-somatic indices (I_G) of females were calculated for each mature specimen using the formula $I_G = 100\ M_G / M_T$. The onset and duration of reproduction seasons were quantified based on the occurrence of individuals with stage-IV ovaries (Table 2). The intensity of reproduction activities was determined by comparing I_G and the proportions of different ovary developmental stages between sampling dates. Relative fecundity of females (F_R) was determined as: $F_R = N_S\ M_G\ M_S^{-1}\ M_E^{-1}$. Mann-Whitney tests were used to test for potential differences in D_E and F_R between the two species. Absolute fecundity of females (F_A) was calculated as: $F_A = N_S\ M_G\ M_S^{-1}$. Analyses of covariance (ANCOVAs) were used to test the differences in F_A between the two species with L_T as a covariate. Chi-square tests (χ^2 test) were used to determine whether the ratio of adult males to females (individuals with gonad stage IV and V were classified as adults, Table 2) varied significantly from a 1:1 ratio within each species and whether adult sex ratios were significantly different between the two species. Logistic regression models were then used to quantify the proportion of mature males and females (stage IV and V, Table 2) at any L_T using binomial maturity data (immature 0, mature 1). L_T at which 50 % of individuals are sexually mature was defined as the mean size of maturity (L_{m50}). A log-likelihood ratio tests were used to test the differences of L_{m50} between sexes within each species (Colonello et al., 2011). Mann-Whitney tests were used to test for potential differences in L_T between males and females within each species. Normality and homogeneity of data was determined using one-sample Kolmogorov–Smirnov test and Levene’s test. All statistical analyses were performed using R version 2.14.0 (R Development Core Team, 2011).

Results

Spawning seasons

R. cliffordpopei and *R. giurinus* showed different onsets of spawning and peaks of spawning seasons. *R. cliffordpopei* spawned from February to June, with a spawning peak occurring in March and April. Spawning activity of *R. giurinus* occurred from April to August with a peak activity during May and June. Specifically, the maturity stage of most ovaries and testes of *R. cliffordpopei* were at stage-I from July to

October, and the sex of individuals was unidentifiable during this period (Table 2). Individuals with stage-IV ovaries appeared first in February. The proportions of stage-IV ovaries were higher than 90 % in March and April, and decreased steadily from May to June. The gonado-somatic indices (IG) of females increased greatly in February and showed the highest values in March and April (Fig. 2). Mean I_G of females was 18.8 % during the peak spawning seasons (March to April). For *R. giurinus*, gonads of most individuals were at stage-I from September to December (Table 2). Mature individuals (those with stage-IV ovaries) were first observed in April and accounted for 76-86 % in May and June. I_G of females increased significantly in April, peaked in June and decreased in July (Fig. 3). Mean I_G of females during the peak spawning seasons (May to June) was 12.4 %.

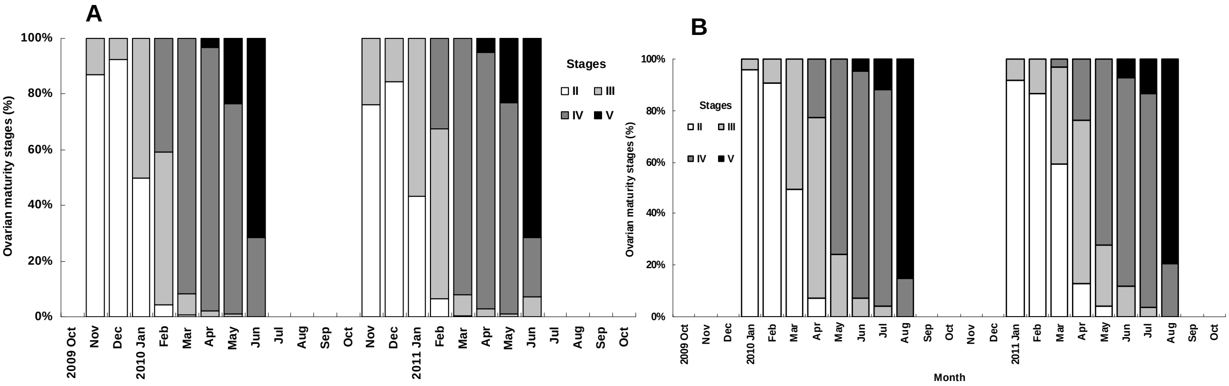

Fig. 2 Monthly changes in the percentage of ovarian maturity stages for (A) *R. cliffordpopei* and (B) *R. giurinus* from October 2009 to October 2011 in Lake Erhai (China). The absence of data indicated that individuals unidentifiable for sexes.

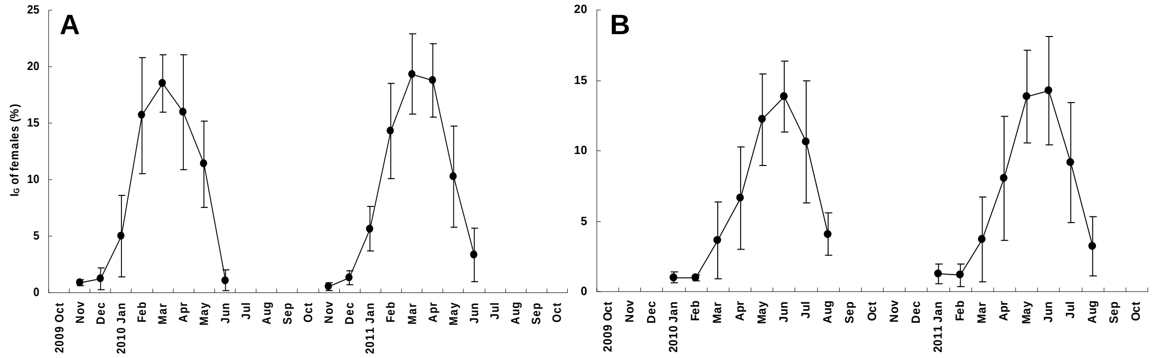

Fig. 3 Monthly changes in the gonado-somatic indices (I_G) of females for (A) *R. cliffordpopei* and (B) *R. giurinus* from Oct 2009 to Oct 2011 in Lake Erhai (China). The absence of data indicated that individuals unidentifiable for sexes. The error-bars represent the standard deviations.

Egg size and fecundity

R. cliffordpopei produced larger eggs and had a lower fecundity than *R. giurinus*. Indeed, diameter of egg (D_E) averaged 613 μm (± 93 SD, n = 2303) for *R.*

cliffordpopei and 470 µm (± 94 SD, n = 1902) for *R. giurinus* (Mann-Whitney test, W = 3967132, $P < 0.001$). Relative fecundity of females (F_R) of *R. cliffordpopei* averaged 2069 eggs g^{-1} (± 3854 SD, n = 61) and was significantly lower than 4597 ± 2368 eggs g^{-1} (± SD, n = 50) of *R. giurinus* (Mann-Whitney test, W = 30, $P < 0.001$). The absolute fecundity of females (F_A) was1581 ± 406 (mean ± SD, n = 61) for *R. giurinus* and 5667 ± 2025 for *R. cliffordpopei* (mean ± SD, n = 50). Irrespective of individual body size, *R. giurinus* showed significantly higher F_A than *R. cliffordpopei* (ANCOVA, df = 1, $F = 103.79$, $P < 0.001$). Moreover, the rate at which F_A increased with increasing L_T was significantly higher in *R. giurinus* (ANCOVA, df = 1, $F = 21.01$, $P < 0.001$, Fig. 4).

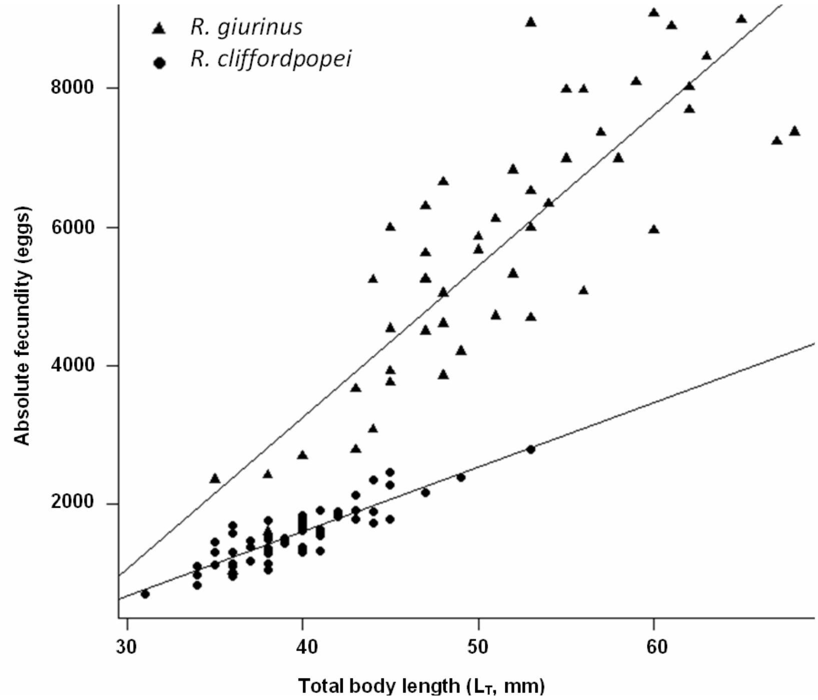

Fig. 4 Comparison of the relationships between total body length (L_T) and absolute fecundity of females (F_A) for *R. cliffordpopei* ($F_A = -2100 + 92.6\ L_T$, r = 0.88, n = 61) and *R. giurinus* ($F_A = -5400 + 218.7\ L_T$, r = 0.86, n = 50) from October 2009 to October 2011 in Lake Erhai (China).

Adult sex ratio

The adult sex ratio differed significantly between the two species (χ^2 test, $\chi^2 = 61.67$, df = 1, $P < 0.001$). The adult sex ratio of *R. cliffordpopei* was significantly biased toward females (1.62 female to 1 male, χ^2 test, $\chi^2 = 112.99$, df = 1, $P < 0.001$, n = 1885), whereas the adult sex ratio of *R. giurinus* was 0.98 female to 1 male and it did not significantly differ from 1:1 (χ^2 test, $\chi^2 = 0.268$, df = 1, $P = 0.605$, n = 2467).

Size at maturity and sexual size dimorphism (SSD)

R. cliffordpopei showed different size at maturity and a strong SSD, whereas there was no significant SSD in *R. giurinus*. Specifically, the mean size of maturity (L_{m50}) of males in *R. cliffordpopei* was 37.6 mm, which was significantly larger than 33.9 mm for females (Likelihood ratio test, df = 2, Chi-squared = 259.82, $P < 0.001$). *R. giurinus* showed similar L_{m50} between males (46.7 mm) and females (47.2 mm, Likelihood ratio test, df = 2, Chi-squared = 4.62, $P = 0.099$) (Fig. 5). Moreover, males of *R. cliffordpopei* had a significantly higher mean L_T (33.4 mm) over females (30.5mm, Mann-Whitney test, W = 324832, $P < 0.001$), whereas L_T was not significantly different between the sexes in *R. giurinus* (38.5 mm for males and 38.9 mm for females, Mann-Whitney test, W = 1046996, $P = 0.392$).

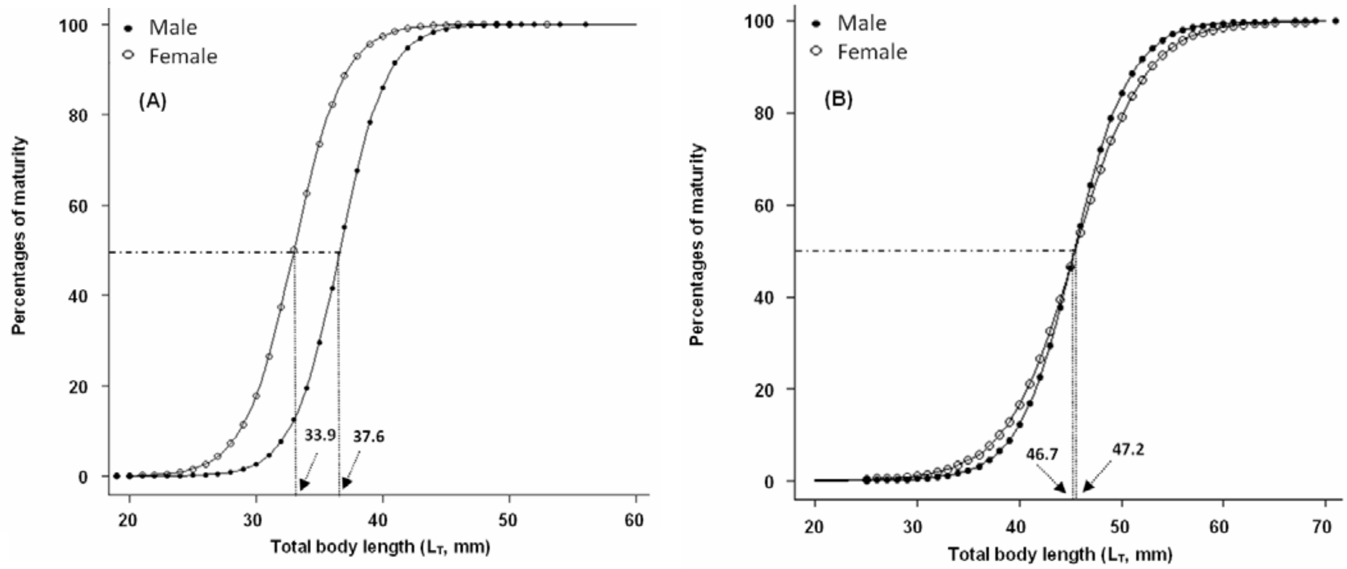

Fig. 5 Comparison of the size of maturity of (A) *R. cliffordpopei* (n = 2293 for females and n = 1819 for males) and (B) *R. giurinus* (n = 2237 for females and n = 2304 for males) from October 2009 to October 2011 in Lake Erhai (China). Mean size of maturity (L_{m50}) was 33.9 mm and 47.2 mm for females and 37.6 mm and 46.7 mm for males of *R. cliffordpopei* and *R. giurinus*, respectively.

Discussion

The present study demonstrated that *R. cliffordpopei* and *R. giurinus* differed in their reproductive biology, notably in the spawning cycles with differences in the onset of spawning and the peak of spawning seasons in Lake Erhai. The spawning cycles of the two species are highly variable in their native range along the middle and lower reaches of the Yangtze River. In Lake Biandangtang, for instance, spawning seasons of both species occur from the middle of April to late July (Zhang, 2005), whereas *R. giurinus* spawns from July to September in Lake Chaohu (coexisting with *R. cliffordpopei*) and Lake Dongting (without *R. cliffordpopei*, Yan & Chen, 2007). When introduced into Lake Fuxian, *R. giurinus* spawned from July to October (Yan & Chen, 2007). Therefore, regionally specific investigations of the spawning cycles of these two species will be critical before any control operations are put into effect. In Lake Erhai, our study was the first to demonstrate that the two species showed clearly different spawning cycles within the same ecosystem outside of their native range, and this is a crucial issue for the timing of deployment of remediation actions (Ling, 2002; Britton et al., 2011). Management operations for fishes may often most effective when implemented pre-spawning because offsprings usually increase several orders of magnitude population size and disperse intensively within the ecosystem (Ludgate & Closs, 2003; Wimbush et al., 2009). Moreover, the removals of adults soon after spawning may result in a population increase in juveniles (e.g. decreased intraspecific competition) rather than their decline. In New Zealand, for example, Ludgate & Closs (2003) revealed that the numbers of young-of-the-year Eurasian perch (*Perca fluviatilis*) in ponds increased markedly after the cannibalistic adult perch were removed after spawning. In the present study, control measures (e.g. eradication actions or physical removals) are probably most effective from September to February for *R. cliffordpopei*, and from January to April for *R. giurinus*.

The adult sex ratio of *R. cliffordpopei* was strongly female-biased whilst *R. giurinus* showed similar numbers of females and males in Lake Erhai. Zhang (2005) found that they showed equitable sex ratio in their native range, suggesting that the sex ratios of the two species at birth are likely to be approximate 1:1. The monthly size frequency of the two species was analyzed (Electronic Supplementary Material, SFig. 1 & SFig. 2) and no evidence of size selectivity by fyke nets was observed. Indeed, the two goby species are congeneric species and show similar males paternal care (Takahashi et al., 2001; Ito & Yanagisawa, 2003). If the paternal caring males were less susceptible to fyke nets than females, then the adult sex ratio of *R. giurinus* would be also female-biased. This was not the case here. Furthermore, benthic fyke nets have been used in previous study (i.e. Zhang (2005) and Yan & Chen (2007)) and the authors did not observe sex-biased adult sex ratio in the two species. Therefore, it is very unlikely that your results on sex ratio were affected by the use of fyke nets. There are several possible mechanisms for the skewed sex ratio of *R. cliffordpopei* in LH. First, differential survival between the sexes caused by local predation of *Channa argus*, an invasive species that was found to be a potential predator of the two goby species in native lakes (Ma & Xie, 1999). The distribution *C. argus* is limited exclusively to LH in Lake Erhai where the species co-occured with *R. cliffordpopei*. Sex-specific predation resulting in biased sex ratio has been widely demonstrated in fishes. For instance, *Rivulus hartii* and *Macrobrachium crenulatum* preyed preferentially on males of *Poecilia reticulate* over females in both field and laboratory experimentations (McKellar et al., 2009; McKellar & Hendry, 2011). Selective predation on males of *R. cliffordpopei* may be due to their conspicuous visual sexual signals (e.g. bright colors (Hurtado-Gonzales et al., 2010)) and/or behaviors (e.g. aggression or mobility (Costantini et al., 2007)). Moreover, since adult males of *R. cliffordpopei* are significantly larger than females, SSD-associated size-selectivity predation on larger males can also result in a higher mortality of males (Nakazawa et al., 2007; Reardon & Thibert-Plante, 2010). Second, sex-specific mortality caused by the costs associated with parental care (Liker & Székely, 2005). Males of the genus *Rhinogobius* are nest-guarders (Takahashi et al., 2001; Ito & Yanagisawa, 2003). Parental care is not only energetically costly but it also increases the vulnerability to predators, which may increase mortality of males of *R. cliffordpopei* in LH (Liker & Sźekely, 2005). Therefore, *C. argus* is probably an effective (and non-native) predator to males of *R. cliffordpopei* in the lake. After an evaluation of its potential effects on non-target species and introduced ecosystem, *C. argus* may be a potential candidate as a bio-control agent in the lake if its ecological impacts are acceptable (Saunders et al., 2010; Britton et al., 2011). Currently, *C. argus* is the only recorded piscivorous fish in the lake with a relatively low abundance. Increasing the abundance of *C. argus* to an appropriate extent by artificially stocking and/or limiting catches by the fishery may be a synergistic operation with the physical removals. As the distribution of *C. argus* is limited exclusively to LH but *R. giurinus* mostly uses PH, further investigations are needed to determine the susceptibility of *R. giurinus* to other sources of natural predation.

Here, *R. cliffordpopei* showed a clearly male-biased sexual size dimorphisms (SSD) while *R. giurinus* showed no SSD. Sexual selection, fecundity selection and inter-sexual resources partitioning selection are the three major selective processes underpinning SSD (Fairbairn, 2007). As a male-guarding species, males of *R. cliffordpopei* may be subjected to a strong sexual selection because the large males often have advantages in acquiring larger and/or better nests (Malavasi et al., 2001; Takahashi et al., 2001). The large males of *R.* sp. DA, for instance, are found to occupy larger nest stones and guard more eggs in each nest over the smaller ones (Takahashi et al., 2001). However, the body size of *R. cliffordpopei* in Lake Biandangtang was similar between the sexes and males of *R. giurinus* were significantly larger than females (Zhang, 2005). The direction and magnitude of these selections often vary substantially among species as well as populations within species in highly different environments (Blanckenhorn et al., 2006). In Lake Erhai, physical removals should give a careful consideration for the male-biased SSD of *R. cliffordpopei* because the effectiveness of physical removals (e.g. intensive gill netting) may vary with different body size of the target fishes (Knapp & Matthews, 1998; Britton et al., 2011). For instance, Knapp & Matthews (1998) found that introduced adult trout were highly vulnerable to gill nets but younger fish were not readily captured until they reached approximately 110 mm. Thus, we suggest that the body size of the smaller *R. cliffordpopei* females is the determinant for minimal mesh size of benthic fyke and seine nets used in the physical removals of *R. cliffordpopei*.

In conclusion, our results demonstrated that the two invasive goby species showed different reproductive characteristics in Lake Erhai. Consequently, the approaches taken in the management of the two species should incorporate these differences and more effective control strategies are recommended in term of timing of implementation of control actions, gear characteristics and/or bio-manipulation programs. Moreover, since the present control operations taken in Lake Erhai have low effective, application of synergistic remediation may be a complementary strategy. Additionally, further investigations on life traits and ecological impacts are imperative for a comprehensive management strategy of these invasive fish species, notably if these invasive species life traits are impacted by climate changes (e.g. Britton et al., 2010).

Acknowledgments

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Comparison of diel activity, feeding rhythm and diet composition of two congeneric goby species: no evidence for temporal and food partitioning

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Abstract

Niche differentiation has been considered as a major mechanism for stable coexistence among competing species within communities. In sub-littoral habitat of Lake Erhai (China), the diel activity, feeding rhythm and diet composition of two congeneric and competing goby species (*Rhinogobius cliffordpopei* and *Rhinogobius giurinus*) were compared to test whether there is niche separation along temporal axis (time partitioning) and/or trophic axis (food partitioning). The results demonstrated that there was no evidence for niche differentiation alongt those two axes. Specifically, the activity of the two species was markedly high during 6:00 - 10:00 and 18:00 - 22:00 in a 24 h periodicity. The diel activity was similar among four seasons within species and was not significantly different between species. They showed two clear feeding periods (i.e. 6:00 - 10:00 and 18:00 - 22:00) in a 24 h periodicity. The feeding intensity was significantly lower in winter compared with the other seasons. There were no significant differences in feeding rhythm between the two gobies. Their diets primarily consisted of macrozooplankton, aquatic insects and shrimp larvae. The diet compositions showed no diel changes but were significantly different among seasons whereby the two gobies consumed more macrozooplankton but less aquatic insects in winter than in the other seasons. The two species displayed similar food compositions in the same time session of a day and in the same season.

Key words: gobies, ecological niche, diel activity, feeding rhythm, temporal partitioning, food partitioning,

Introduction

One of the central goals in ecological research is to explain the stable coexistence of ecologically similar species within an ecosystem (Chesson 2000; Amarasekare 2003; Murrell & Law 2003; Kylafis & Loreau 2011). The principle of competitive exclusion, called Gause's law, predicts that one species would eventually outcompete and displace the other one when they compete for the same critical resources within an environment (Hardin 1960). However, a striking pattern in nature is not to be the case in large number of ecologically similar species and several explanations have been put forward, of which niche differentiation has been developed for a long time (e.g. Schoener 1974; Ross 1986) and considered as a major mechanism of coexistence among competing species in ecological communities (Jones et al. 2001; Amarasekare 2003; Kylafis & Loreau 2011). Species niche differentiation basically involves spatial axis, trophic axis, temporal axis or a combination of them (Chesson 2000; Kronfeld-Schor & Dayan 2003). In heterogeneous environments, habitat or microhabitat specialization (spatial axis) and food resource partitioning (trophic axis) have been thought to be the most frequent mechanisms of coexistence among ecologically similar species (Chesson 2000; Jones et al. 2001; Amarasekare 2003; Murrell & Law 2003). Although theory suggests that diel temporal partitioning is also an important strategy mediating ecological interactions and facilitating coexistence among co-occurring competitors, its role in structuring communities has never been a strong focus in ecology (Kronfeld-Schor et al. 2001; Richards 2002; Kronfeld-Schor & Dayan 2003). Currently, a growing number of studies have accumulated and provided empirical evidences of temporal partitioning between competing species such as ants (Albrecht & Gotelli 2001), bats (Adams & Thibault 2006), mice (Jones et al. 2001; Gutman & Dayan 2005) and birds (Veen et al. 2010). In fish assemblages, however, the role of the niche separation along temporal axis has received much less attention (Alanärä et al. 2001).

The activity (locomotory activity and feeding activity) of most animal species changes with a 24 h periodicity, which has usually evolved to cope with the time structure, abiotic and/or biotic conditions in the environments of living organisms (Kronfeld-Schor & Dayan 2003; Roll et al. 2006). These activity patterns have substantial ecological implication and evolutionary significance, one of which is the promotion of coexistence through temporal partitioning at the diel scale (Richards

2002; Kronfeld-Schor & Dayan 2003). The differences in diel activity patterns can mitigate competition via decreasing the resources overlap (i.e. exploitation competition) and/or avoiding the direct confrontation (i.e. interference competition) (Kronfeld-Schor & Dayan 1999; Kronfeld-Schor et al. 2001). Despite the fact that there have been empirically evidences of the niche separation along habitat (Sone et al. 2006; Hernaman & Probert 2008; Horinouchi 2008) and food resource axes (Kakareko et al. 2005; Borza et al. 2009; Borcherding et al. 2012) in several sympatric goby species, the information on niche differentiation along time axis is extremely scarce for co-occurring goby species.

Rhinogobius giurinus (Rutter 1897) and *Rhinogobius cliffordpopei* (Nichols 1925) are frequent small fishes in the middle and lower reaches of the Yangtze River. They are two ecological-similar species with similar life histories and diets in those native lakes (Xie et al. 2000a; Xie et al. 2005; Zhang 2005; Wu & Zhong 2008). The two species have become the most dominant benthic fish species in the lake since they were introduced inadvertently into Lake Erhai in 1961 (Du & Li 2001, Guo et al. 2010a). Our previous revealed that the abundance of *R. cliffordpopei* is highest in littoral habitat (LH, water depths less than 6 m) and lowest in profundal habitat (PH, water depth range from 12 to 20 m), whereas the abundance of *R. giurinus* is highest in PH and lowest in LH (Guo et al. 2012a). Moreover, we demonstrated that they showed food partitioning in LH but no evidence of food partitioning was found in PH (Guo et al. 2012b). The abundances of the two species are intermediate in sub-littoral habitat (SH, water depth ranges 6 to 12 m, Guo et al. 2012a). However, little is know of their feeding behavior and food compositions in this habitat. In the present study, therefore, diel and seasonal activity, feeding rhythm and food composition of the two gobies were compared in SH. The specific objectives are (i) to determine their diel and seasonal activity, feeding rhythm and food composition, and (ii) to test whether there is temporal partitioning (i.e. differences in diel and seasonal activity and feeding rhythm) and/or food partitioning (i.e. differences in diet compositions) between the two species within a habitat.

Materials and methods

Study sites

Lake Erhai (105°5-17′ E, 23°35-58′ N) is a freshwater lake in Yunnan-Guizhou Plateau of China, southwest of China. Its water surface area is about 250 km^2 with maximum water depth of 21 m. The sample sites of this study located in sub-littoral habitat (SH, Fig. 1). In this area, water depths ranged 6 to 10 m, few submerged macrophytes were found, substrata structures were mixture of submersed macrophyte detritus and soft mud (Guo et al. 2012a). The fish species in SH are *R. giurinus* and *R. cliffordpopei*, *Pseudorasbora parva* (Temminck & Schlegel 1846), *Hypseleotris swinhonis* (Günther 1873), *Hemiculter leucisculus* (Basilewsky 1855) and *Channa argus* (Cantor 1842). *Rhinogobius giurinus* and *R. cliffordpopei* are the most dominant fishes in this habitat (Guo et al. 2012a).

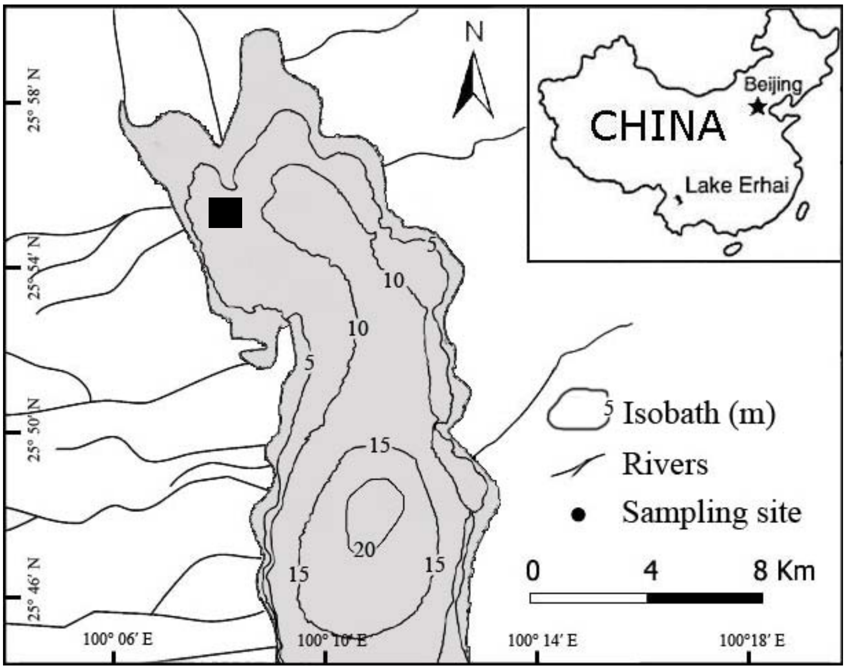

Fig. 1 Sample areas (■) of *R. giurinus* and *R. cliffordpopei* in sub-littoral habitat of Lake Erhai (China) in the four seasons of 2011.

Fish sampling

R. cliffordpopei and *R. giurinus* were sampled using benthic fyke nets in spring (April), summer (July), autumn (October) and winter (January) in 2011. The fyke nets comprised a trunk stem with twenty traps (0.60 × 0.62 × 0.35 m), two end-traps and two end-pockets (Guo et al. 2012a). Total length of the net was 15 m, including 12 m of traps (0.6 m each), 2 m of end traps (1 m each) and 1 m of end pockets (0.5 m each). The stretched mesh size was 4 mm. In each season, gobies were collected once very two hours in 24 hours of a day. Five nets were used for each time. The nets fist deployed at 7:00, retrieved at 8:45 and deployed again at 9:00 for a next round. From the second time, the sample site was moved randomly and was proximately two hundred meters away from the previous one. For each time, all gobies in the end pockets of the nets were collected and counted. If the individuals collected were more than thirty, a random sub-sample (about 30 individuals) were took and preserved in 8 % formalin for two weeks and then transferred to 75% ethanol for storage. Otherwise, all individuals were preserved.

Gut content analyses (GCA)

Total body length (L_T) and body mass (M_T) of each specimen for GCA were measured to the nearest mm and 0.01 g, respectively (Table 1). Since there was no clear stomach for the two species, the fore-gut, which was defined as the section of intestine from the oesophagus to the posterior end of the first loop, was sampled for diet analyses (Xie et al. 2000a; Xie et al. 2005). The fore-guts were identified using a dissecting microscope. After removing surface ethanol by blotting them on tissue paper, the non-empty guts were weighed to the nearest 0·01 mg, then food contents were removed and the emptied guts were reweighed. The food contents in non-empty guts were preserved in 2 ml plastic tube with 75 % ethanol. Food items were identified to the lowest possible taxonomic level under a dissecting microscope. The weight of Cladocera and Ostracoda were calculated as 0.023 mg per individual. Copepoda was calculated as 0.014 mg per individual. The larva of Copepoda was calculated as 0.003 mg per individual (Zhang 2005). The weight of fish eggs, plant materials and unidentified items were roughly equal to their volumes (specific gravity was assumed to be 1). Shrimp larvae, aquatic insects, Gastropoda and fish larvae were weighted to the nearest 0.1 mg (Hyslop 1980). For each food category, frequency of occurrence (%O), percentage of number (%N), percentage of weight (%W) were calculated as formulas: $\%Oi = 100\ Oi / \sum_1^n Oi$, where O*i* is the number of the guts that contain food category *i*; $\%Ni = 100\ Ni / \sum_1^n Ni$, where N*i* is the number of food category *i*; $\%Wi = 100\ Wi / \sum_1^n Wi$, where W*i* is the weight of food category *i* (Hyslop 1980). Food compositions of the two goby species were finally expressed using index of relative importance (%IRI) that was calculated as formula: $\%IRI = 100\ IRIi / \sum_1^n IRIi$, where $IRIi = (\%Ni + \%Wi) / \%Oi$ (Assis 1996).

Table 1 Total body length (TL, mean ± tandard deviation (SD)) and body weight (BW, mean ± SD) of *R. giurinus* and *R. cliffordpopei* used for gut content analysis in the four seasons of 2011in Lake Erhai (China).

	R. giurinus		*R. cliffordpopei*	
	TL (mm)	BW (g)	TL (mm)	BW (g)
spring	37.7 ± 7.9 (n=125)	0.45 ± 0.23	36.1 ± 4.9 (n=112)	0.44 ± 0.19
summer	40.3 ± 6.6 (n=118)	0.52 ± 0.27	27.4 ± 3.2 (n=132)	0.18 ± 0.08
autumn	33.6 ± 4.4 (n=134)	0.36 ± 0.15	30.7 ± 5.4 (n=127)	0.27 ± 0.11
winter	35.4 ± 4.5 (n=103)	0.40 ± 0.21	32.5 ± 2.7 (n=98)	0.32 ± 0.15

Data calculation and statistical analyses

The diel and seasonal activity were determined using the percentage of catch per unit effort (PCPUE, %) of benthic fyke nets, which was calculated as: PCPUE (%) = 100 × the number of individuals caught in a given two hours per net / the number of individuals caught in 24 hours of day per net. The differences in PCUPE (log (x+1) transformed) among seasons and species were tested using Mixed-Effects Models with seasons and species as fixed factors and time of the day as a random factor. Because seasons had significant effects on CPUE (Table 15), one-way ANOVAs were subsequently used to test the differences in PCPUE between seasons within species. Multiple comparisons among means were conducted using Tukey’s HSD *post-hoc* test when significant difference was identified by one-way ANOVAs.

The diel and seasonal feeding rhythm were determined using the percentage of empty gut (PEG) and weight of gut content (WGC). PEG was calculated as: FEG (%) = 100 number of empty guts/ number of total guts. WGC was calculated as: WGC (%) = 100 food weight of non-empty gut / eviscerated weight of the body. Mann-Whitney test were used to test the differences in PEG among seasons within species, and between the two species within the same season. The differences in WGC (log (x+1) transformed) among seasons and species were tested using Mixed-Effects Models with seasons and species as fixed factors and time of the day as a random factor. The subsequent analyses were the same as PCPUE.

The diel and season changes of diet compositions were determined using %IRI of each food category. Because the sample sizes for each sample time were small for %IRI analysis, the samples were pooled in morning (6:00 - 11:59 h), afternoon (12:00

- 17:59 h), early night (18:00-23:59 h) and late night (24:00 - 5:59 h). Thus, the %IRI was calculated for those four time sessions of a day. Chi-square test (χ^2 test) was used to the differences in %IRI among the time sessions in each season, and between four seasons within species. χ^2 test was used to test the differences in %IRI of each food category among seasons within species, and between species within the same time session.

Normality and homogeneity of data was determined using one-sample Kolmogorov–Smirnov test and Levene's test. All statistical analyses were performed using R version 2.14.2 (R Development Core Team 2012).

Results

Diel and seasonal activity and feeding rhythm

These two species had two peaks of activity in 24 hours of a day (6:00 - 10:00 and 18:00 - 22:00) and showed similar activity patterns among seasons. The percentage of catch per unit effort (PCPUE, %) of the two species increased sharply at 6:00 and 18: 00, and dropped at 10:00 and 22:00 in all the four seasons (Fig. 2). Although Mixed-Effects Models revealed that seasons had significant effects on PCPUE (Mixed-Effects Models, P <0.001, Table 2), the one-way ANOVAs suggested that PCPUE were not significantly different among seasons ($F_{3,236}$ = 0.781, P = 0.506 for *R. giurinus* and $F_{3,236}$ = 0.844, P = 0.471 for *R. cliffordpopei*).

Table 2 Mixed-Effects Models used to test the differences in the percentage of catch per unit effort (PCPUE, log (x+1) transformed) of *R. giurinus* and *R. cliffordpopei* among seasons and species with seasons and species as fixed factors and the time of a day as a random factor in 2011 in Lake Erhai (China).

Sources	Num. d.f.	Den. d.f.	*F*	*P*
Seasons	3	454	8.179	<0.001
Species	1	454	0.886	0.347
Time	1	10	1.011	0.338
Species × Seasons	3	454	1.077	0.358
Species × Time	1	454	0.588	0.444
Seasons × Time	3	454	7.176	<0.001
Species × Seasons × Time	3	454	2.791	0.040

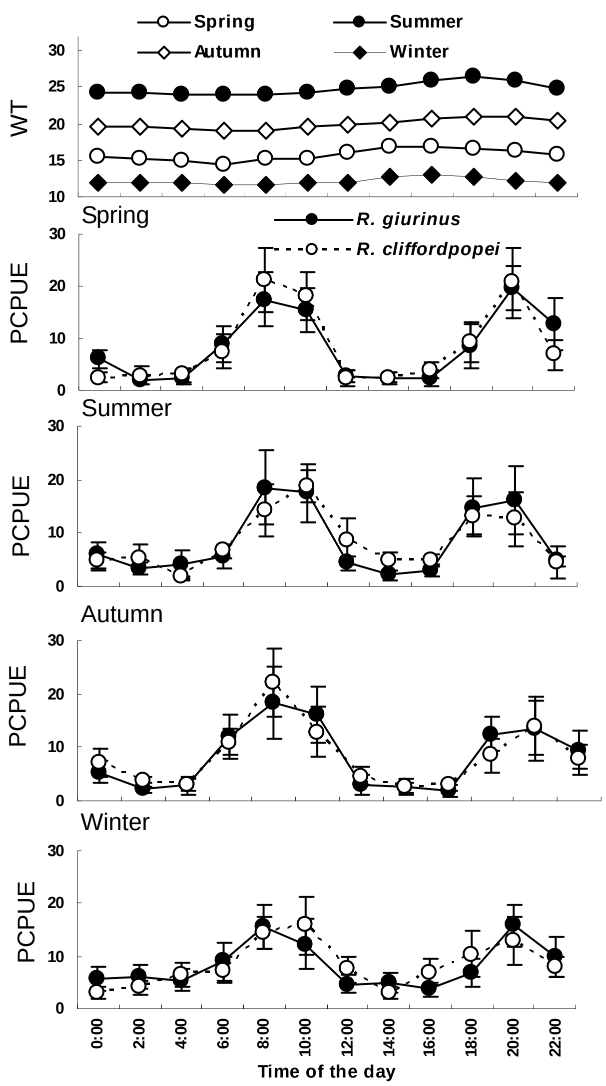

Fig. 2 Diel changes in water temperature (WT, °C) and the percentage of catch per unit effort (PCPUE, %) of *R. giurinus* and *R. cliffordpopei* in the four seasons of 2011 in Lake Erhai (China). Bars are standard deviations.

They showed two main feeding periods in 24 hours of a day (i.e. 6:00 - 10:00 and 18:00 - 22:00) (Fig. 3 & Fig. 4). The diel feeding rhythm was similar among the season but feeding intensity was significantly different among seasons (Fig. 3 & Fig. 4). The percentage of empty gut (PEG) was clearly low from 6:00 to 10:00 and from 18:00 to 22:00 in the four seasons (Fig. 3). In contrast, the weight of gut content (WGC) was markedly high from 6:00 to 10:00 and from 18:00 to 22:00 (Fig. 3). PEG of both species were significantly lower in winter (Mann-Whitney test, $P < 0.05$), but similar among the other seasons (Mann-Whitney test, $P > 0.1$, Fig. 4 & Table 3). WGC varied significantly among seasons (Mixed-Effects Models, $P < 0.001$, Table 4). *R. giurinus* showed the lowest WGC in winter and highest in spring (Tukey's HSD, $P < 0.001$), but showed similar values in summer and autumn (Tukey's HSD $P = 0.863$, Fig. 5). For *R. cliffordpopei*, WGC was lowest in winter (Tukey's HSD, $P < 0.001$) and higher in autumn than in spring (Tukey's HSD, $P = 0.013$), whereas it was similar between spring and summer (Tukey's HSD, *P* = 0.574), and between summer and autumn (Tukey's HSD, *P* = 0.238, Fig. 4).

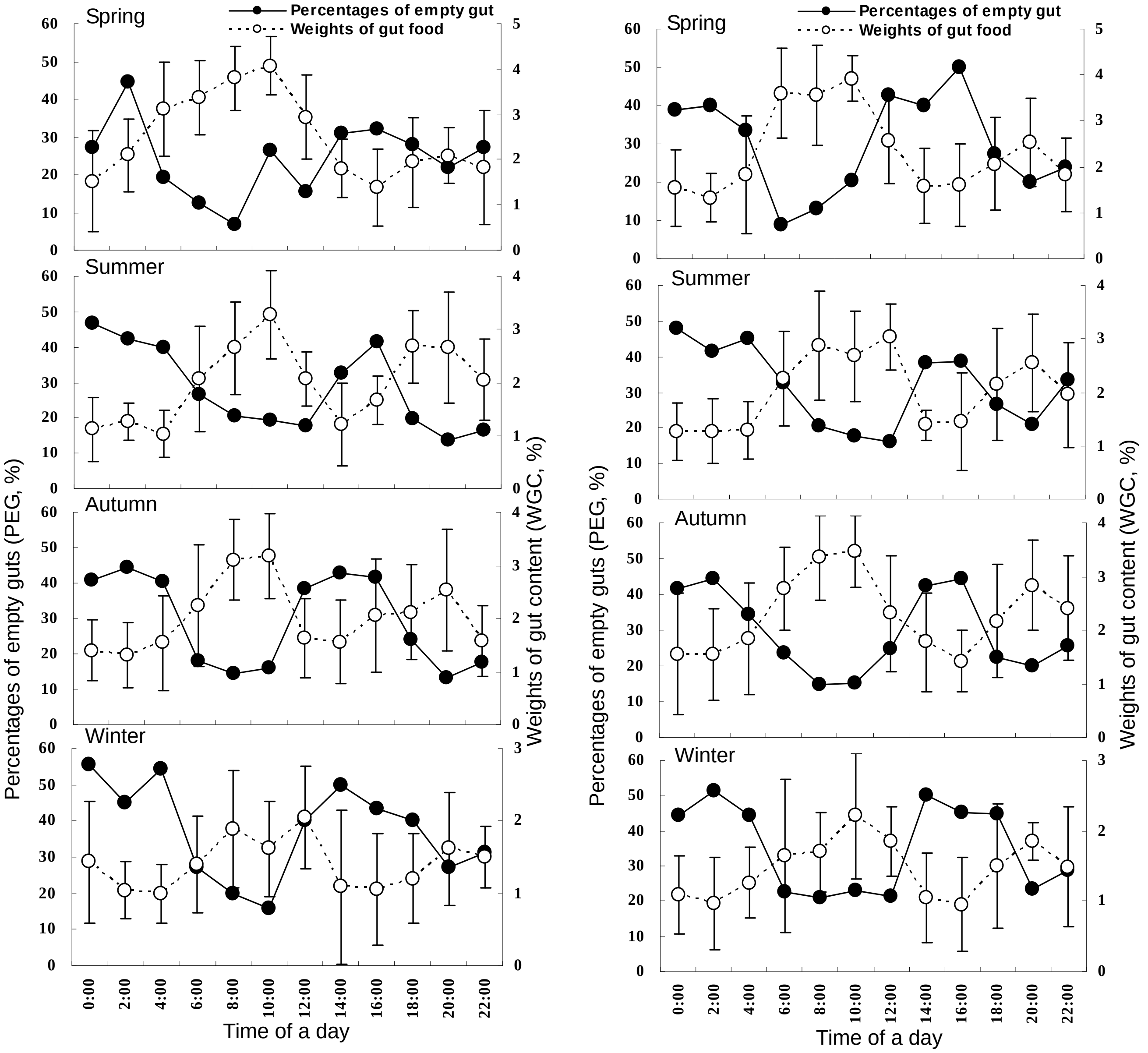

Fig. 3 Diel changes in percentages of empty gut (PEG, %) and weights of gut content (WGC, %) of (left) *R. giurinus* and (right) *R. cliffordpopei* in the four seasons of 2011 in Lake Erhai (China). Bars are standard deviations.

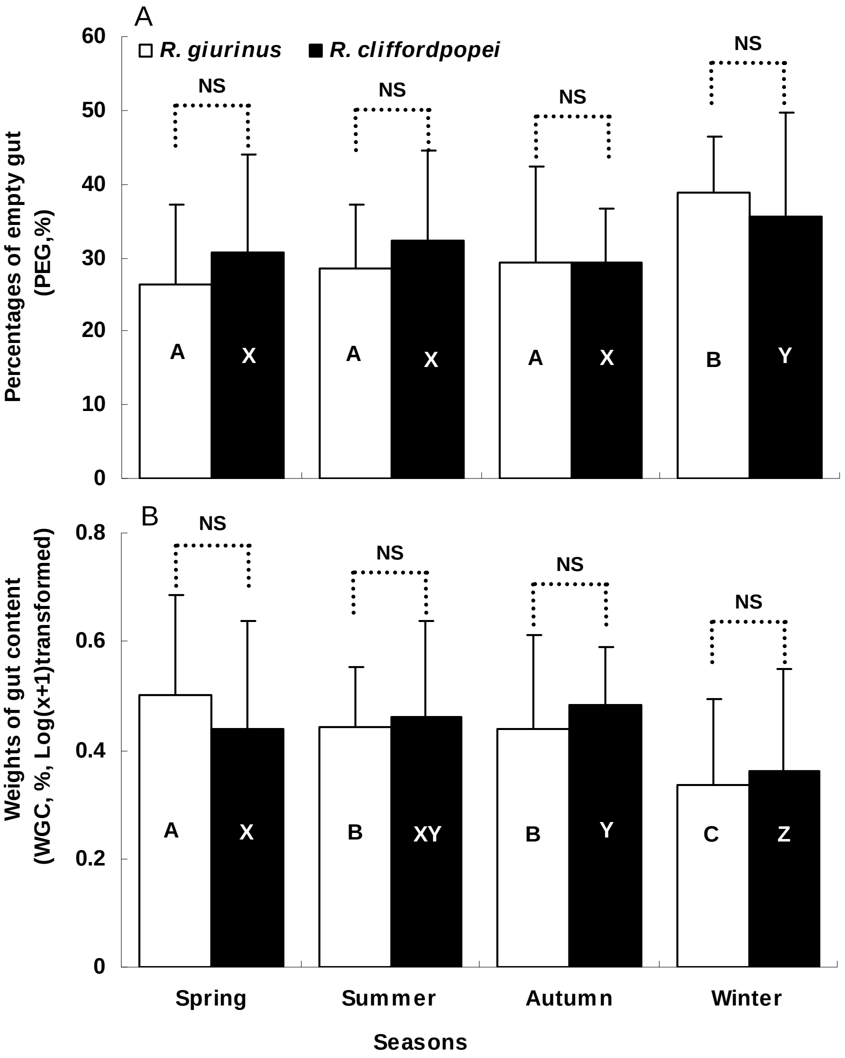

Fig. 4 Seasonal changes in (A) percentages of empty gut (PEG, %) and (B) weights of gut content (WGC, %, log(x+1) transformed) of (left) *R. giurinus* and (right) *R. cliffordpopei* in 2011 in Lake Erhai (China). Bars are standard deviations. The means with different letters in histograms are significantly different among seasons within species. The means with NS are not significantly different between species within the same season

Table 3 Mann-Whitney test used to test the differences in percentages of empty gut (PEG) of *R. giurinus* and *R. cliffordpopei* among seasons within species in 2011 in Lake Erhai (China).

		winter		spring		summer	
		V	*P*	V	*P*	V	*P*
R. giurinus	spring	74	0.003				
	summer	65	0.042	36	0.850		
	autumn	77	<0.001	23	0.398	35	0.791
R. cliffordpopei	spring	65	0.042				
	summer	66	0.034	31	0.569		
	autumn	72	0.007	35	0.893	54	0.266

Table 4 Mixed-Effects Models used to test the differences in weights of gut food (WGC, log (x+1) transformed) among seasons and species with seasons and species as fixed factors and the time of a day as a random factor.

Sources	Num. d.f.	Den. d.f.	*F*	*P*
Seasons	3	1495	46.812	<0.001
Species	1	1495	0.392	0.531
Time	1	10	0.656	0.437
Species × Seasons	3	1495	4.517	0.004
Species × Time	1	1495	0.009	0.925
Seasons × Time	3	1495	3.577	0.014
Species × Seasons × Time	3	1495	0.841	0.472

Diel and seasonal changes in diet compositions

Diets of the two gobies primarily consisted of macrozooplankton, aquatic insects, and shrimp larvae (Fig. 5). Their food compositions (%IRI) were similar among the four time sessions of a day in all the seasons ((χ^2 test, $P > 0.1$, Fig. 5 & Table 5) but differed significantly among seasons (χ^2 test, d.f. = 27, $\chi^2 = 41.898$, $P = 0.034$ for *R. giurinus* and $\chi^2 = 40.727$, d.f. = 27, $P = 0.0437$ for *R. cliffordpopei*, Fig. 6). The %IRI of macrozooplankton was higher in winter but that of aquatic insects was lower in winter compared with the other seasons (χ^2 test, $P < 0.05$, Fig. 6 & Table 6). Diet compositions were similar among spring, summer and autumn within species (χ^2 test, $P > 0.1$, Fig. 6 & Table 6).

Table 5 Chi-square test (χ^2 test) used to test the differences in food compositions (%IRI) of *R. giurinus* and *R. cliffordpopei* among the four time sessions of a day within species in fours seasons of 2011 in Lake Erhai (China).

		χ^2	d.f.	*P*
R. giurinus	spring	32.906	27	0.200
	summer	18.657	27	0.882
	autumn	33.537	27	0.179
	winter	25.796	27	0.529
R. cliffordpopei	spring	21.147	27	0.779
	summer	29.959	27	0.316
	autumn	35.142	27	0.135
	winter	24.137	27	0.623

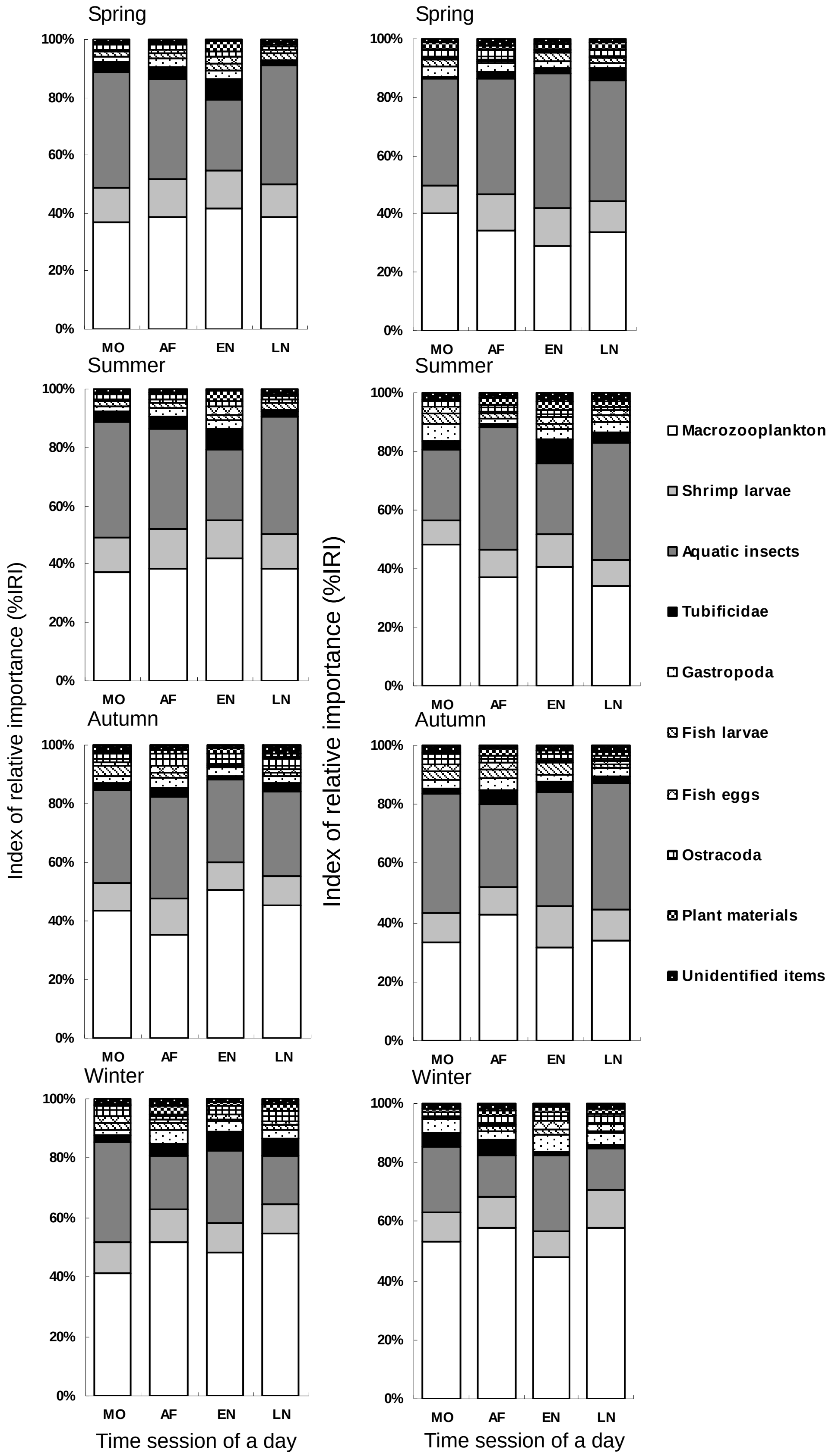

Fig. 5 Changes of diet compositions (%IRI) of (left) *R. giurinus* and (right) *R. cliffordpopei* at morning (MO), afternoon (AF), early night (EN) and late night (LN) of a day in the four seasons of 2011 in Lake Erhai (China).

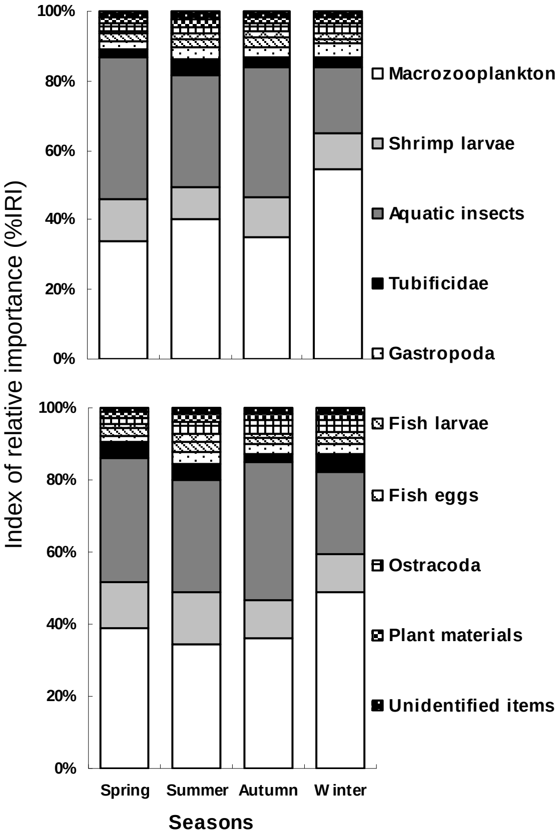

Fig. 6 Seasonal changes in diet compositions (%IRI) of (top) *R. giurinus* and (bottom) *R. cliffordpopei* of 2011 in Lake Erhai (China)

Table 6 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) of *R. giurinus* and *R. cliffordpopei* among seasons within species in 2011 in Lake Erhai (China).

		spring			summer			autumn		
		χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
R. cliffordpopei	summer	5.271	9	0.810						
	autumn	9.207	9	0.418	4.005	9	0.911			
	winter	22.182	9	0.008	17.434	9	0.014	19.602	9	0.021
R. giurinus	summer	8.360	9	0.498						
	autumn	10.775	9	0.291	4.384	9	0.884			
	winter	34.174	9	<0.001	17.434	9	0.014	18.609	9	0.028

Comparison of activity, feeding rhythm and food composition between species

The diel and seasonal activity were similar between species (Mixed-Effects Models, *P* = 0.347, Table 2). Feeding rhythms were similar between the two species, i.e. they showed similar PEG (Mann-Whitney test, *P* = 0.233, *P* = 0.410, *P* = 0.722 and *P* =

0.964 for spring, summer, autumn and winter, respectively, Fig. 3 & Fig. 4) and WGC (Mixed-Effects Models, $P = 0.531$, Fig. 3 Fig. 4 & Table 3).

The diet compositions (%IRI) varied slightly between them in the same time session of a day (χ^2 test, $P > 0.05$, Fig. 5 & Table 6) and in the same season (χ^2 test, $P > 0.1$, Fig. 6 & Table 7).

Table 6 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) between *R. cliffordpopei* and *R. giurinus* in each time session (morning, afternoon, early night and late night) in the four seasons of 2011 in Lake Erhai (China).

	R. cliffordpopei											
	Morning			Afternoon			Early night			Late night		
R. giurinus	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Spring	10.339	9	0.324	2.55	9	0.979	5.85	9	0.755	4.591	9	0.868
Summer	5.709	9	0.768	3.298	9	0.951	15.290	9	0.083	4.137	9	0.902
Autumn	7.185	9	0.618	8.984	9	0.439	5.581	9	0.781	7.756	9	0.559
Winter	3.732	9	0.928	3.180	9	0.957	10.336	9	0.324	5.830	9	0.756

Table 7 Chi-square test (χ^2 test) used to test the differences in diet compositions (%IRI) between *R. cliffordpopei* and *R. giurinus* in the four seasons of 2011 in Lake Erhai (China) in 2010.

	R. giurinus		
R. cliffordpopei	χ^2	d.f.	*P*
spring	13.093	9	0.158
summer	10.566	9	0.306
autumn	2.008	9	0.991
winter	6.130	9	0.726

Discussion

Diel and seasonal activity and feeding rhythm

Diel activity patterns of fishes are highly diverse and plastic among different species as well as different cohorts or individuals within species (Reebs 2002). Those activities are molded by heredity factors (e.g. phylogenetic histories, Roll et al. 2006; developmental factors, Reebs 2002) and by epigenetic selective forces (e.g.

environmental factors, Reebs 2002). For goby species, their locomotory activity is mostly nocturnal (e.g. *Neogobius melanostomus* (Pallas 1814), *Neogobius kessleri* (Günther 1861), *Neogobius fluviatilis* (Pallas 1814), Erös et al. 2005; *Pomatoschistus minutus* (Pallas 1770), Thetmeyer 1997; Ehrenberg & Ejdung 2008) and only *Gobiusculus flavescens* (Fabricius 1779) exhibits a diurnal activity rhythm (Thetmeyer 1997). In Lake Erhai, the diel activity patterns of these two goby species were typically crepuscular (6:00 - 10:00 and 18:00 - 22:00). Moreover, our study revealed that the feeding rhythm of the two gobies was also distinct crepuscular and coincided well with the locomotory activity. A similar phenomenon is observed in *G. flavescens*, of which the timing of swimming activity was closely coupled with foraging behavior in laboratory (Thetmeyer 1997). There are some gobies, however, show inconsistent patterns between locomotory activity and feeding rhythm (e.g. *P. minutus*, del Norte-Campos, & Temming 1994; *G. flavescens*, Thetmeyer 1997; *N. fluviatilis*, Grabowska et al. 2009). Although some studies suggest that gobies are typical visual feeders and their highest feeding rates are in twilight hours (del Norte-Campos & Temming 1994; Kanou et al. 2005), feeding rhythms of gobies show greatly variability among different species (e.g. *Neogobius gymnotrachelus* (Kessler 1857) is crepuscular, Grabowska & Grabowski 2005; *Acanthogobius flavimanus* (Temminck & Schlegel 1845) is nocturnal and crepuscular, Kanou et al. 2005; *N. fluviatilis* shows no clear changes in feeding activity in a 24 h periodicity, Grabowska et al. 2009). These variations probably resulted from phylogenetic differences between species and/or different environmental factors (e.g. temperature, light intensity, prey availability, predation risk, etc., Reebs 2002; Stoner 2004).

In the present study, the diel activity and feeding rhythm of the two gobies was similar among the four seasons, whereas the feeding intensity was significantly low in winter. Many fishes change locomotory activity and/or feeding behaviors in different seasons (Reebs et al. 2002). For instance, G*alaxias argenteus* (Gmelin 1789) is continuously active in summer but changes to be nocturnal in winter (David & Closs 2001). One of the most proximate and direct causes for the changes of activity and feeding rhythms in winter is low temperature (Reebs et al. 2002; Hasler et al. 2009). In Lake Erhai, the mean temperature is 12.1 °C in winter (Fig. 2). The lower feeding intensity in winter probably resulted from the low water temperature. Changes of feeding intensity with seasons were also observed in *Rhinogobius brunneus* ‘orange’, which showed higher stomach fullness but less empty guts in spring and summer than autumn (Rusuwa et al. 2009).

Diel and seasonal changes in diet compositions

Although the two goby consumed a variety of food items, their main diets were macrozooplankton (Cladocera and Copepoda) and aquatic insects (Chironomidae larvae) in SH of Lake Erhai. This is well consistent with results observed in the native lakes along the middle and lower reaches of the Yangtze River (Xie et al. 2000a; Xie et al. 2005; Zhang 2005). In littoral habitat (LH) and profundal habitat (PH) of the lake, the similar body-sized individuals of both species primarily consume similar diets with those in SH (Guo et al. 2012b). The diet compositions did not display diel changes in each of the four seasons but varied significantly among different seasons in this study. In Biandantang Lake, *R. giurinus* also showed seasonal differences in diet compositions, i. e. the importance of chironomid larvae and ostracoda was higher in winter and spring than in summer and autumn (Xie et al. 2000a). Several other gobies were observed to change diets seasonally (e.g. *N. melanostomus* consumed primarily chironomids in spring but the mixture of amphipods and molluscs in the summer and autumn, Borza et al. 2009; *R. brunneus* preyed more insect but less algal in spring and summer than in autumn, Rusuwa et al. 2009). Those changes were largely owing to seasonal variations of availability and/or suitability of food resources (Xie et al. 2000a; Borza et al. 2009; Rusuwa et al. 2009). In Lake Erhai, the density of benthos (Mollusca, oligochaeta and aquatic insects) was significantly higher in spring and summer than in autumn and winter, whereas the density of zooplankton showed the inverse pattern of benthos (unpublished data). The diets consumed by the two gobies coincided with the food availability in different seasons. Most invasive goby species are opportunistic feeders with broad diet spectrum and their food items usually can closely match with available food resources in different habitats (Kakareko et al. 2005; Grabowska et al. 2009; Polačik et al. 2009; Rusuwa et al. 2009) and/or times (Xie et al. 2000a; Grabowska et al. 2009; Rusuwa et al. 2009; Borcherding et al. 2012).

No evidence for temporal and food partitioning

In SH, our results demonstrated that there was no temporal and food partitioning between the two species. Generally, niche differentiation along spatial dimension is important more important than trophic dimension and then temporal dimension

(Schoener, 1974). For fish assemblages, Ross (1986) found that about 57% niche showed the greatest separation by food, 32% by habitat, and 11% time among 37 studies that concurrently examined the niche differentiation along habitat, food and temporal axes (Ross, 1986). In Lake Erhai, habitat segregation is probably the main mechanism for the coexistence of the two competing gobies (Guo et al. 2012a). Several gobies were evidenced to partition food resources when they become quite abundant within an ecosystem (Kakareko et al. 2005; Borza et al. 2009; Borcherding et al. 2012). In Lake Erhai, the two gobies display food partitioning in LH where the ecological similar species (e.g. *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Hypseleotris swinhonis* (Günther, 1873)) are highly abundant (Guo et al. 2012a; Guo et al. 2012b). The densities of the two gobies and other ecological similar species are low in SH and the food resources may be enough for them. For temporal partitioning, Kronfeld-Schor & Dayan (2003) argued that it would be a viable mechanism for reducing resource competition when the shared limiting resources differ between activity times (particularly for those species whose prey populations show activity patterns) or when the limiting resources are renewed within the time involved in the separation. In this study, macrozooplankton may display diel vertical migration that can cause changes of food resources at bottom among different activity times (Hay 2003; Williamson et al. 2011). However, aquatic insects do not show diel variations and these two main food items seems to not show diel renewed patterns. Therefore, further laboratory investigations are needed to provide a better understanding of their feeding behaviors and competitive interactions under various food resource levels (e.g. limited *vs* saturated) and/or different changes of food resources (e.g. stimulating diel vertical migration of macrozooplankton or seasonal variation of aquatic insects).

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Appendix

Outreach activities during PhD study

Publications

[1] **Zhiqiang Guo**, Xiaoming Zhu, Jiashou Liu, Dong Han, Yunxia Yang, Shouqi Xie & Zeqiao Lan (2012). Effects of dietary protein level on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, *Acipenser baerii* ♀ × *A. gueldenstaedtii* ♂. *Aquaculture* 338-341: 89-95.

[2] **Zhiqiang Guo**, Xiaoming Zhu, Jiashou Liu, Dong Han, Yunxia Yang, Shouqi Xie & Zeqiao Lan (2011). Dietary lipid requirement of juvenile hybrid sturgeon, *Acipenser baerii* ♀ × *A. gueldenstaedtii* ♂. *Journal of Applied Ichthyology* 27: 743-748.

[3] **Zhiqiang Guo**, Zhongjie Li, Jiashou Liu, Fengyue Zhu & H. A. C. C. Perera (2012). Status of reservoir fisheries in China and their effects on environments. In *Tropical and Sub-tropical Reservoir Limnology in China (B.P. Han and Z. Liu (eds.) Monographiae Biologicae* 91: 259-276.
DOI 10.1007/978-94-007-2007-7_15.

[4] Han Dong, Xie S, Zhu X., Yang Y. & **Guo Z**. (2010). Growth and hepatopancreas performances of gibel carp fed diet containing low levels of aflatoxin B1. *Aquaculture Nutrition* 16: 335-342.

Research Projects

[1] Scientific innovation fund (20,000 RMB, 2011) for postgraduate student granted by Graduate University of Chinese Academy of Sciences. The proposal title is: "*Status of Fishes Biodiversity and Evaluation of Rank of Endangered Native Fishes in Basin of Lake Erhai, China*".

[2] Program Cai Yuanpei 2011-2013 funded by Chinese Scholarship Council and University of Toulouse. This program is established by the French Ministry of Foreign and European Affairs (MAEE) and Higher Education and Research (MESR) and Chinese Ministry of Education (CME). The proposal title is: *Biological Invasions of Fishes in Lakes of Yunnan-Guizhou Plateau of China*.