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Temporal variability of larval drift of tropical amphidromous gobies along a watershed in Réunion Island

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Abstract

Freshwater gobies of tropical islands are amphidromous: adults reproduce in rivers and larvae passively drift down to the sea immediately after hatching. Describing the phenology of this larval drift is essential to understand the population dynamics of amphidromous gobies and to develop ecologically based recommendations for managing the watersheds. The larval drift patterns of two amphidromous gobies of Réunion Island, *Sicyopterus lagocephalus* and *Cotylopus acutipinnis*, were studied on a monthly basis for one year using plankton nets at two sites of the Mât River, located 20 km and 7 km from the river mouth. Genetic analyses showed that larval assemblages were dominated at 90 % by *S. lagocephalus* although the proportion of *C. acutipinnis* increased during early summer. Our findings highlighted a marked larval drift peak in full austral summer. The diel larval drift dynamic was described using periodic linear models. A diel pattern was only observed downstream with a peak few hours after sunset. Finally, this study presents a new approach improving standard methods for sampling and analyzing larval drift of amphidromous species.

Keywords: diadromy, downstream migration, river continuum, Sicydiinae, tropical islands

Résumé

Les gobies dulçaquicoles des îles tropicales sont amphidromes : les adultes se reproduisent dans les rivières et, immédiatement après l'éclosion, les larves dérivent passivement jusqu'à la mer. La description de la phénologie de la dérive des larves en eau douce est indispensable à la compréhension de la dynamique des populations de ces gobies amphidromes et pour préconiser des mesures de gestion des bassins versants adaptées à leur écologie. Les patrons de dérive des larves de deux espèces de gobies amphidromes de l'île de La Réunion, *Sicyopterus lagocephalus* et *Cotylopus acutipinnis*, ont été décrits mensuellement pendant une année à l'aide de filets planctoniques au niveau de deux sites de la rivière du Mât, situés respectivement à 20 km et 7 km de l'embouchure. Les analyses génétiques ont montré que les assemblages larvaires étaient dominés à 90 % par *S. lagocephalus.* Cependant, la proportion de *C. acutipinnis* était plus importante en début d'été. Nos résultats ont mis en évidence que la dérive des larves avait lieu principalement pendant l'été austral. La dynamique journalière de dérive des larves a été décrite à l'aide de modèles périodiques linéaires. Un patron journalier n'a été observé qu'en aval avec un pic quelques heures après la tombée de la nuit. Enfin, cette étude présente une approche originale, basée sur l'amélioration des méthodes standards d'échantillonnage et d'analyse des données de dérive des larves des espèces amphidromes.

Mots clefs: diadromie, migration de dévalaison, continuité écologique, Sicydiinae, îles tropicales

Introduction

Diadromy is a life history strategy involving migrations between freshwater and marine biomes (Myers, 1949). Within diadromy, amphidromy is a subcategory in which the longest part of the individuals growing period as well as their reproduction, occur in freshwater habitats (Manacop, 1953). Newly hatched larvae of amphidromous species immediately drift down to the sea where they grow for months (Radtke et al., 1988; Bell et al., 1995). At the end of their larval stage, they return to estuaries and settle in rivers to complete their life cycle as juveniles and adults (McDowall, 2007). Amphidromy is widespread among fish species (McDowall, 1988) especially in tropical islands where most indigenous fishes are amphidromous (Keith, 2003). The highly dispersive capacities of amphidromous fishes have been demonstrated for several species (Chubb et al., 1998; Berrebi et al., 2005). The drift of their larvae in rivers corresponds to the first part of this dispersal phase. Benefits of dispersal are numerous: avoiding the intergenerational competition, reducing the risks of inbreeding, and spreading larvae away from temporally unsuitable habitats (Burgess et al., 2015). The fact that rivers can become temporally unsuitable for fish species has been particularly discussed in the tropical context where cyclonic floods, droughts during the dry season, or even volcanic eruptions can occur and lead to temporary extirpation of fish populations (McDowall, 2010). In the context of such unpredictable conditions, the dispersal stages of amphidromous species contribute to explain the success of this life strategy in tropical islands (McDowall, 2010). On the other hand, the risk of mortality during the larval stage is supposed to be huge. For example, Artzrouni et al. (2014) evaluated that the survival probability throughout the whole larval stage of *Sicyopterus lagocephalus* may range from 10^{-4} to 10^{-5}. Even during the short period of freshwater larval drift, Bell (2009) estimated that mortality could be as high as 50 % per hour for *Sicydium* species. In this context, it is likely that amphidromous species have developed different strategies for reducing the mortality of their larvae when drifting in freshwater streams and rivers (McDowall, 2009).

Superimposed on these natural risks of mortality, drifting larvae of amphidromous fishes also face numerous anthropogenic threats. Alteration of the river continuity and the hydrological regimes due to dams can reduce the access to growing spawning habitats and/or their availability (Brasher, 2003; Cooney and Kwak, 2013). Larval survival can also be severely impaired by dams since an important part of the drifting larvae can be entrained within the diverted waters (Benstead et al., 1999). Moreover, alteration of downstream flow regime can increase the risk of river droughts, the duration of these droughts, and can also result in the closure of the river mouth (Lill et al., 2013). The consequences of these flow alterations can lead to a total loss of the larvae drifting downstream (McDowall, 1995). Despite the fact that harmful effects of dams on drifting larvae have been documented for years, this critical period of the amphidromous fish life cycle has not received much attention from managers (Benstead et al., 1999; Lagarde et al., 2015). It may be due to the inefficiency of existing methods (i.e. intake screens, behavioral barriers…) to avoid migrating small-sized larvae of being entrained within diverted waters (Larinier et al., 1994).

In Réunion Island, a 2 500 km² volcanic island in the southwestern Indian Ocean, the freshwater fish assemblages are dominated by two amphidromous gobies, *Sicyopterus lagocephalus* and *Cotylopus acutipinnis*. These two species present an important cultural value for local populations since the individuals entering estuaries at the end of their marine larval life are traditionally fished (Aboussouan, 1969; Bell, 1999). Once in freshwater, post-larvae transform into juveniles and colonize the watersheds up to the headwaters thanks to their efficient climbing abilities (Blob et al., 2006; Schoenfuss and Blob, 2007). The abundances of *S. lagocephalus* adults gradually decrease from estuaries to upper habitats located at approximately 30 km upstream and at more than 600 m above sea level (a.s.l.), whereas the abundances of *C. acutipinnis* adults are equally distributed throughout the rivers (Teichert et al., 2014a). Reproduction of both species occurs during the warmer months in middle and upper reaches but *C. acutipinnis* is capable of spawning at lower temperatures than *S. lagocephalus* (Teichert et al., 2014b, 2016). Thus reproduction in upstream reaches extends from December to May when the temperature is higher than 19 °C whereas reproduction can occur during the whole year in downstream reaches (Teichert et al., 2014b). Both species present similar fecundities ranging from 13 500 to 14 900 oocytes per gram (wet weight) of female and can spawn several times during the reproductive season with an inter-spawning interval of approximately one month (Teichert et al., 2014b, 2016). Females lay eggs clutch under pebbles and rocks in runs and riffles (Teichert et al., 2013a). After egg fertilization, the male guards the nest until larvae hatch (Teichert et al., 2013b). Immediately after hatching, larvae, which are at larval stage 1 according to Valade et al. (2009) and Ellien et al. (2016), passively drift down to the sea. Their only activity is a swim up - sink down behavior and they die if they do not reach the sea within few days after hatching (Valade et al., 2009).

The main objective of this study was to establish the spatiotemporal patterns in drifting larvae of *S. lagocephalus* and *C. acutipinnis*. As the number of drifting larvae should reflect both direct (i.e. on the larvae), and indirect (i.e. on the spawning population) disturbances, the ultimate aim was to evaluate the advantages of surveying drifting larvae for assessing the biotic integrity of tropical rivers.

Material and methods

Sampling sites

The study was conducted in two sites along the watershed of the Mât River on the east side of Réunion Island (Fig. 1). This torrential river flows over 36.3 km from its spring at 2 850 m a.s.l. Important hydrological variations are recorded between the dry and the rainy seasons, this latter spanning from November to April, and during which short and intensive cyclonic floods with peak discharge up to three hundred times the mean annual discharge can occur (Robert and Davy 1988). One sampling site (Site 1, Fig. 1b) was located at 385 m a.s.l. and 20.1 km from the river mouth. The river width at this site was approximately 14 m. A dam is present immediately downstream but the diversion of water was not operating during the sampling period. The other site (Site 2, Fig. 1b) was located downstream to the main tributary (Fleurs Jaunes River), at 120 m a.s.l. and 6.7 km from the river mouth. The river width at this site was approximately 19 m. A dam is located 150 m upstream to Site 2. Water diversion due to this dam has not been operative for years; however, the dam disturbs the upstream migration of most diadromous fish and crustacean species and only *S. lagocephalus, C. acutipinnis*, freshwater eels (*Anguilla* spp.) and “chevaquines” shrimps (*Atyoida serrata*) can usually reach upstream habitats thanks to their climbing capacities. The riverine habitat was very similar in both sites with no canopy cover and running water over a substratum mainly composed of small boulders and cobbles. Such substratum composition is suitable for *S. lagocephalus* and *C. acutipinnis* to build their spawning-nests (Teichert et al., 2013a), and eggs clutches have been observed under boulders and cobbles in both sites (R. L., personal observations). This observation is consistent with a previous study, which observed eggs clutches in different places of the Mât River from 7 to 20 km to the sea (Teichert et al., 2013a). It is thus likely that spawning grounds of Sicydiinae are present from

Site 2 to the upper limit of presence of those species, which is several kilometers upstream to Site 1.

Sampling plan

At Site 1, a total of 14 sampling sessions were performed from May 2013 to June 2014. At Site 2, 13 sampling sessions were performed from July 2013 to July 2014. The frequency of the sampling sessions was approximately monthly, depending on meteorological conditions and staff availability. During the study, sunrise ranged from 05:30 to 6:55 and sunset from 17:45 to 19:05. At the beginning of each sampling session, an estimate of the river discharge (Q_{river}, $m^3.s^{-1}$) was performed. The river cross section was divided into a minimum of ten vertical subsections. In each subsection, the water velocity was measured using a current meter (Mini current meter type M1, SEBBA Hydrometry) at one to seven locations depending on the water depth. The discharge of each subsection was estimated by multiplying the subsection area by its mean water velocity. The river discharge corresponded to the sum of the discharges of all subsections. The number of subsections, the position of each measurement, and the method used to calculate the mean velocity for each subsection, were based on the recommendation of the standard NF EN ISO 748 (2007).

A gauging station located 3.8 km downstream of the junction of the Mât and the Fleurs Jaunes Rivers (Fig. 1b) recorded the instantaneous discharge every 6 min during the entire study except during a cyclonic flood event on 2 January 2014 and for the 45 following days. This flood presented a peak discharge of approximately 30 times the mean annual discharge (Julien Bonnier, Office de l'eau Réunion, personal communication). Data of this gauging station are available online (www.eaureunion.fr, accessed in October 2015). Due to this flood event, data of the gauging station were only available from May to December 2013 and from February to July 2014. For all the sampling sessions during which the gauging station recorded data, the instantaneous flow estimated every 6 min at this station remained within 4 % (sd = 1 %) of the mean diel flow recorded. The only exception was on 27 November 2013, when a small flooding event started at approximately 18:30. Sampling was stopped for safety reasons and only the first three two-nets samples obtained from 9:22 to 15:46 were kept for analyses. As a consequence, for all the sampling sessions except one, the river discharge estimated manually at each sampling site was considered to be constant over the whole duration of the sampling session.

Sampling procedure

At each site, one sampling session consisted in two conical nets set every two to four hours over a minimal period of 24 hours. The nets were similar to those developed by Bell (1994, 2007) for quantitative drift sampling in small streams. Their mouth diameter was 0.12 m, cod end diameter 0.06 m, length 1 m, and mesh size 80 µm. One of the two nets was fixed in the center of the stream flow, whereas the other one was randomly positioned along the river cross section. The distances between the two nets ranged between 0.3 m and 6.3 m (mean ± sd = 2.1 m ± 1.3) in Site 1, and between 0.2 m and 16.8 m (5.0 m ± 4.1) in Site 2. Each net was set separately but the second net was always set within a period never exceeding 10 min after setting of the first net. Using two nets quasi simultaneously, one of the nets having a random position in the river, allowed to take into account the eventual heterogeneity in the spatial distribution of larval flow along the cross section of the river. In order to save time, if no larvae were observed in the first two two-nets samples, only two to five two-nets samples were performed.

Each net was placed facing upstream at about 50 % of the water depth. It was attached with a removable fixation fixed on an iron rod hammered into the substratum. Each net was immersed between one and 20 min (D_{net}), depending on the water velocity, in order to filtrate a volume of about 2 m^3 (mean ± sd = 2.4±0.8). As the nets were not equipped with a current meter, two measures of water velocity (v_1 and v_2) were performed in front of the mouth of each net, immediately after being set and before being removed, with a mini current meter type M1, SEBBA Hydrometry. The sampled volume (V_{net}) was estimated according to equation (1):

$$(1) \quad V_{net} = \frac{v_1 + v_2}{2} \times \pi \times r^2 \times D_{net}$$

where v_1 and v_2 are the instantaneous measures of water velocities (m.s^{-1}) before and after the sampling, r is the radius of the net (i.e. 0.06 m) and D_{net} is the sampling duration (s).

After removing the net, the solid elements were immediately transferred from the cod end into a 2 L bottle filled with water from the river in order to keep the larvae alive. The larvae were counted alive as their movements facilitate their detection, sorting, and identification (Bell, 2007). First, the sample was kept still for at least 2 hours in the bottle to let vegetal and mineral debris to settle down. Larvae remained in the water column thanks to their behavior of alternately actively swimming upward and passively sinking (Bell and Brown, 1995; Valade et al., 2009). Larvae were then collected by carefully pouring the contents of the bottle on an 80 µm mesh sieve. The pouring was stopped before the overflow of vegetal and mineral debris. The water was returned to the bottle in order to repeat the decanting procedure twice. Larvae were transferred to a counting dish. All the larvae in the counting dish were counted by removing each individual using a 15 µL micropipette (Bell, 2007). The total number of larvae (N_{larvae}) was the sum of all the larvae captured at the end of the three decants process. For each sample, the larvae were placed in a labeled 1 mL Eppendorf® and fixed with 95 % alcohol solution. This live counting procedure was performed within a period never exceeding 15 hours after sampling. This delay was assumed to not affect the number of living larvae as the first mortality of Sicydiinae larvae usually occurs after approximately 24 hours spent in freshwater (Valade et al., 2009; Iida et al., 2010). This assumption was confirmed by visual observations of active larvae 15 hours after sampling.

The instantaneous larval flow (q_{larvae}, ind.s^{-1}), corresponding to the number of larvae per second drifting through the river cross section, was calculated. The use of larval flow instead of larval density (i.e. number of larvae per filtrated volume) was preferred because values of larval flow are not affected by dilution effect when the discharge increase (Barthem et al., 2014). Comparison of larval drift intensities between sites and seasons can thus be performed even if discharge varies. The instantaneous larval flow was calculated for each net according to equation (2):

$$(2) \qquad q_{larvae} = \frac{N_{larvae}}{V_{net}} \times Q_{river}$$

where N_{larvae} is the number of larvae caught in the net, V_{net} is the sampled volume (m^3) and Q_{river} is the river discharge (m^3.s^{-1}).

Differences in larval flow between each net of the two-net samples were tested for the whole data set using a paired Student *t*-test. When no significant difference was detected, the mean value of both nets was used as an estimate of the instantaneous larval flow.

Genetic identification of species

No morphological criteria discriminate the larvae of *S. lagocephalus* and *C. acutipinnis* (Teichert, 2012). The larvae of both species present the same size, shape, color and a similar repartition of their chromatophores. Criteria commonly used for morphological identification of amphidromous fish larvae (Lindstrom, 1999; Bell, 2007; Maeda and Tachihara, 2010; Jarvis and Closs, 2015) are thus not useful for *S. lagocephalus* and *C. acutipinnis*. Identification at the species level can only be based on DNA analysis.

A sub-sample of 768 larvae was randomly selected among the dates covering the whole period during which the number of larvae caught was greater than 30. As a result, the number of larvae selected per date ranged from 33 to 57. Total genomic DNA was extracted from each whole larva using DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Genotyping was performed using the microsatellite locus SIC122, which expresses specific alleles for each species (at 166 pb for *C. acutipinnis* and from 194 to 242 pb for *S. lagocephalus*; Hoareau et al., 2009). The forward primer was indirectly fluorochrome labeled (6-FAM) as described in Schuelke (2000), and polymerase chain reactions (PCR) were performed in a total volume of 10 µL: 5 µL of MasterMix Applied 2x (Applied Biosystems, Foster City, CA, USA), 2.25 µL of demineralized water, 0.25 µL (1 µM) of forward primer to which a M13 tail was added at the 5'end, 0.25 µL (10 µM) of reverse primer, 0.25 µL (10 µM) of a fluorophore-labeled universal M13 primer and 2 µL of genomic DNA. The thermocycling program was as follow : 1 x (5' at 94°C), 7 x [30'' at 94°C, 30'' at 62°C (-1°C each cycle), 30'' at 72°C], 40 x [30'' at 94°C, 30'' at 55°C, 30'' at 72°C], 8 x [30'' at 95°C, 30'' at 56°C, 30'' at 72°C], 1 x (5' at 72°C). Amplicons were run on an ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA, USA) and allele size was determined using Genemapper v 4.0 (Applied Biosystems, Foster City, CA, USA).

Diel variations of larval flow

In each site, the diel variations of instantaneous larval flow were analyzed using periodic linear regressions as proposed by Bell (2007). Only the sampling sessions during which more than 100 larvae were caught and spanning over a minimum of 24 hours were used to fit the regressions (four dates for Site 1 and seven dates for Site 2). Regarding to the very low proportion of *C. acutipinnis* larvae captured during most of these dates (see results), it appeared impossible to consider this species separately in the analyses. Indeed, the estimated number of *C. acutipinnis* larvae per sampling session was always less than 100 for all the dates. Periodic linear regressions are frequently used to model predictable cyclical events and allow the use of time as a continuous variable rather than a series of arbitrary categories (Bliss, 1958; Bell, 2008). This is particularly appropriate for our study as the time interval between two successive two-net samples (from two to four hours) and the number of two-net samples per sampling session (from nine to 12) were not constant between sampling sessions.

For each site, a mixed periodic model was adjusted with varying mesor and regression coefficients for each sampling session in order to integrate the variability of larval flow between dates (3):

$$(3) \quad Y = \beta_0 + \beta_1 \sin(R_h) + \beta_2 \cos(R_h) + r(1 + \sin(R_h) + \cos(R_h) | Session)$$

where Y represents the instantaneous larval flow, R_h is the radian transformed hour of the day (hour x $2\pi/24$), β_0 is the mesor (i.e. mean response of the biological trait over cycles), β_1 and β_2 are the regression coefficients and r is the matrix of individual effects for the sampling sessions. Residuals of regressions were plotted as a function of a theoretical normal distribution (quantile-quantile plot) to verify the assumption of normality. The significance of parameters was calculated based on the F-statistic using the Satterthwaite's approximation method for fixed part and on likelihood ratio tests for random part (χ^2 tests). The peak position (P, hour of the day) was determined for each periodic linear model from equation (4):

$$(4) \quad P = 365 \times \frac{arctan\left(\frac{\beta_1}{\beta_2}\right) + QC}{2\pi},$$

where QC is a quadrant correction ($QC = 0$ if β_1 and β_2 are both positive, $QC = \pi$ if β_1 is positive and β_2 negative or if both are negative and $QC = 2\pi$ if β_1 is negative and β_2 positive).

This peak was considered significant even if one part of the periodic model (i.e. β_1 or β_2) was not significant because cycles cannot be expressed in less than two dimensions (Bell, 2007).

As the random part appeared significant for each site (see results), periodic linear models were adjusted for each of the 11 sampling sessions (four for Site 1 and seven for Site 2) according to equation (5):

$$(5) \qquad Y = \beta_{n,0} + \beta_{n,1}\sin(R_h) + \beta_{n,2}\cos(R_h)$$

where Y represents the instantaneous larval flow, R_h is the radian transformed hour of the day (hour x 2π/24), $\beta_{n,0}$, $\beta_{n,1}$ and $\beta_{n,2}$ are respectively the mesor and the regression coefficients for the sampling session "n". The significance of each parameters $\beta_{n,1}$ and $\beta_{n,2}$ was calculated based on the F-statistic.

Diel abundance of larvae and seasonal flow

The diel abundance of larvae corresponds to the number of larvae drifting through the river cross section during 24 hours. Since the intervals between two-nets samples within a sampling session were not constant, and as the larval flow varied with time during a sampling session, a simple arithmetic mean of larval instantaneous flow (q_{larvae}) did not appear adequate to estimate the diel abundance of larvae. Diel abundances of larvae (A_{larvae}, ind.day^{-1}) were thus obtained by integrating the instantaneous larval flow values obtained over 24 hours (Fig. 2) according to equation (6):

$$(6) \qquad A_{larvae} = \frac{\int_{\bar{t}_1}^{\bar{t}_n} \bar{q}_{larvae}(\bar{t})\,\mathrm{d}t}{D_{total}} \times 24 \times 3600$$

where n is the number of two-nets samples obtained during a sampling session, $\bar{t}$ is the mean time of sampling (s) of the two-nets sample ((t_{net1}+t_{net2})/2; with t_{net1} and t_{net2} the median times of sampling for each net, respectively) ranging from $\bar{t}_1 = 0$ to $\bar{t}_n = D_{total}$, D_{total} corresponds to the total sampling duration (s) of the sampling session and $\bar{q}_{larvae}$ is the mean value of instantaneous larval flow (ind.s^{-1}) of the two-nets sample ((q_{larvae_net1}+ q_{larvae_net2})/2).

All statistical analyses were performed with the open source R v. 3.1.2 software (R Development Core Team 2013), implemented with the lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2015) packages for the mixed model analyses and tis (Hallman, 2014) package for diel abundance and seasonal flow estimations.

Results

Sampling effort and relative proportion of species

Drifting larvae were caught on 29 May and 20 June 2013 and between 12 December 2013 and 19 May 2014 in Site 1 (Table 1). In Site 2, drifting larvae were caught during the whole sampling period except on 19 August 2013. The nets set in Site 1 (n = 182) and Site 2 (n = 239) allowed to collect a total of 3 222 and 3 796 larvae, respectively. The number of larvae captured per sampling session ranged from 0 to 1 842 in Site 1 and from 0 to 736 in Site 2.

The measured river discharge ranged from 0.6 to 3.6 $m^3.s^{-1}$ in Site 1 and from 2.7 to 11.6 $m^3.s^{-1}$ in Site 2 (Table 1). Maximum values of river discharges were observed during the first sampling sessions after the cyclonic flood and then decreased the following months.

The genetic identification of larvae at the species level was successfully achieved for 746 individuals (between 33 and 56 larvae per sampling session) among the 768 larvae randomly selected. A total of 668 larvae (90 %) presented the alleles of *S. lagocephalus* whereas 78 (10 %) presented the allele 166 of *C. acutipinnis* (Table 1). In Site 1, the mean proportion of *S. lagocephalus*, weighted by the diel abundance of larvae, was 94 %. The lowest proportion of *S. lagocephalus* (79 %) was observed on 12 December 2013. In Site 2, the mean proportion of *S. lagocephalus*, weighted by the diel abundance of larvae, was 92 %. The lowest proportion of *S. lagocephalus* (57 %) was observed on 27 November 2013 and proportions of 85 % and 78 % were observed on 5 May 2014 and on 2 June 2014, respectively.

Diel variations of larval flow

The two sites considered altogether, instantaneous larval flows ranged from 0 to 2 275 $ind.s^{-1}$ and did not significantly differ between each net of the two-net samples (paired Student *t*-test, n = 210, $t_{208} = -1.40$, $P = 0.16$). The orthogonal relationship of the log transformed larval flow estimated from the fixed net (net 1) and from the randomly positioned net (net 2) illustrated that the larval flow was similar across the river's section (Fig. 3). As a consequence, the mean value ($\overline{q}_{larvae}$) of both nets was used for the following analyses.

The instantaneous larval flow ($\overline{q}_{larvae}$) varied along the day with diel coefficients of variation varying from 17.2 % to 141.4 % for Site 1 and from 41.5 % to 300.0 % for Site 2. The diel pattern of larval flow generally showed a unique peak, sometimes two peaks (Fig. 4).

In Site 1, the part of variance explained by fixed periodic parameters β_1 and β_2 was not significant, reflecting the absence of consensual diel periodic effect (Table 2, Fig. 4a). Oppositely, the fixed periodic parameters estimated for the Site 2 explained a significant part of larval flow variability and predicted a consensual peak of larval flow at 22:30 (Table 2, Fig. 4b). The random periodic parameters included in each model explained a significant part of variance (χ^2 test, df = 3, $P < 0.05$ in both sites), reflecting that the hour of the day had an effect on the larval flow for some sampling sessions.

The periodic linear models adjusted separately for each sampling session were consistent with the observed values of larval flow (Fig. 4). For Site 1, the part of variance explained by the parameters $\beta_{n,1}$ and $\beta_{n,2}$, used to estimate the location of the peaks, was only significant for two sampling sessions over the four. The two significant predicted peaks on 21 February and 28 March 2014 occurred during the afternoon (i.e. 12:30 and 13:35, F-test, $P < 0.05$, Fig. 4a). For Site 2, predicted peaks occurred between 19:55 and 00:10, except on 16 December 2013 when the predicted peak occurred at 12:30. The part of variance explained by the parameters $\beta_{n,1}$ and $\beta_{n,2}$, used to estimate the location of the peaks, was significant (F-test, $p < 0.05$, Fig. 4b) except on 28 October 2013.

Diel abundance of larvae and seasonal larval flow

The diel abundance of larvae ranged between 0 and 17 x 10^6 larvae.day^{-1} for Site 1 (Fig. 5a) and between 0 and 44 x 10^6 larvae.day^{-1} for Site 2 (Fig. 5b). In both sites, a marked maximum was observed in January-February. A good concordance was observed in both sites between the highest diel abundances of larvae and the highest values of river discharge.

Discussion

Temporal and spatial dynamics of larval flow

A weak but significant diel pattern of larval drift was observed for both species of Sicydiinae in the downstream site, showing a consensual peak of larvae few hours after sunset. The same pattern has been reported in similar tropical contexts for several amphidromous gobies (Lindstrom, 1998), and among them Sicydiinae species (Bell, 2007) or for crustaceans (March et al., 1998; Benstead et al., 1999). Nocturnal drift may correspond to a strategy for reducing predation (Lucas and Baras, 2001). The poor swimming abilities of Sicydiinae larvae (Bell and Brown, 1995) suggest that drifting few hours after sunset is more probably due to a synchronism of hatching instead of an active behavior of the larvae. Two hypotheses could explain this synchronism in hatching time. First, it could be the result of a preferred spawning time of adults during the day. The short incubation duration of Sicydiinae eggs, approximately three days according to Lindstrom (1998), makes plausible the hypothesis that spawning events over a short period of time can lead to the larvae hatching at a given time. For example, if adults spawn mainly at sunset, larvae could hatch approximately at sunset three days later. Synchronicity between diel spawning behavior of adults and diel larval drift has been observed in other species, such as *Osmerus mordax* (Bradbury et al., 2004). These authors correlated hatching of eggs to the decrease of luminosity at sunset. However, the eggs clutches of Sicydiinae are buried under cobles, so that luminosity in the nest is probably constantly low and makes this explanation less likely. The second hypothesis is that hatching could be provoked by the guarding male by poking and fanning the eggs as suggested by Maeda and Tachihara (2010).

Conversely, no clear diel pattern of larval drift was observed upstream. However, two significant peaks of larval drift, calculated for two different dates, were found to occur in the afternoon. The absence of clear diel larval drift pattern in upstream areas has already been observed for several amphidromous species such as *Rhinogobius brunneus* by Iguchi and Mizuno (1990), or Palaemonidae and Atyidae shrimps by March et al. (1998). Two types of explanations were given for the lack of diel pattern of drifting larvae in upper reaches. First, Iguchi and Mizuno (1990) suggested that the higher complexity of hydrodynamics in upstream reaches could delay the drift of larvae due to their retention in eddies. Secondly, March et al. (1998) made the hypothesis that the absence of predators in upstream reaches could offset the advantage of nocturnal drift. The river sampled in Réunion Island presents a highly turbulent flow from head waters to the estuary, it is thus unlikely that the different patterns of larval drift could be related to differences in hydraulic conditions between upstream and downstream sites. The hypothesis of March et al. (1998) seems more consistent regarding the structure of fish populations in the rivers of Réunion Island. Indeed, most indigenous predators such as *Eleotris* spp., *Kuhlia* spp., and *Agonostomus* sp. are present in downstream reaches only (Keith et al., 2006). *Atyoida serrata* is a common crustacean species of upstream reaches, it is a detritivore species feeding mainly on organic matter and algae (Keith et al., 2006). So, the only predatory species commonly observed upstream are *Anguilla* spp. which are probably not predators of newly hatched larvae due to their very small size. Assuming that the mean flow velocity is 50 $cm.s^{-1}$ between the two studied sites which are approximately 13 km apart, the time larvae would take for passively drifting from Site 1 to Site 2 would be approximately 7 hours. Thus, larvae caught in higher abundances during the afternoon at the upstream site would reach the downstream reaches of the river, where most of their predators are present, at night. However, estimation of the contribution of larvae from Site 1 to the observed dynamics in Site 2 is much complicated due to the continuous presence of spawning grounds between both sites.

A very strong seasonal peak of larval drift, matching the period of highest flow, was observed in both studied sites. The period during which drifting larvae were observed corresponded to the reproductive season that was expected based on ovaries maturation of *S. lagocephalus* (Teichert et al., 2014b) and *C. acutipinnis* (Teichert et al., 2016). However, the larval drift peaks were more intense than what could be speculated from these previous studies. High flow conditions thus appeared to strongly influence the larval drift of Sicydiinae in Réunion Island as observed for amphidromous gobies in Hawaiian rivers (Lindstrom, 1998; Way et al., 1998). This synchronism between high flow and high larval drift could be a strategy to reduce the drifting time and thus the risk of mortality at this stage (Bell, 2009) due to starvation (Moriyama et al., 1998; Iguchi and Mizuno, 1999), developmental constraints (Bell and Brown, 1995) or predation. Predation is probably also lower during high flow due to the increased turbidity limiting the detection of larvae by predators (Araujo-Lima et al., 2001).

Relative proportion of species

The relative proportion of larvae observed was only partially in agreement with the spatial repartition of adults of *S. lagocephalus* and *C. acutipinnis* and what is known about their reproductive biology. However, the overall proportion of *S. lagocephalus* larvae caught during this study (90 %) was consistent with the common proportion of adults of this species in rivers of Réunion Island. Indeed, fish monitoring programs have demonstrated that this species generally represents more than 80 % of the total abundance of Sicydiinae and *C. acutipinnis* 20 % only (Mérigoux et al., 2012). However, the proportion of *S. lagocephalus* adults is known to decrease from downstream to upstream reaches (Teichert et al., 2014a). Moreover, although the fecundity and inter-spawning intervals of both species are similar, *C. acutipinnis* is able to spawn at lower temperatures than *S. lagocephalus* (Teichert et al., 2014b, 2016). As a consequence, a lower proportion of *S. lagocephalus* larvae was expected upstream as well as at the beginning and the end of the reproductive season, when the mean diel water temperatures are below 19°C. But in this study, the proportions of *S. lagocephalus* larvae were approximately the same at both sites, and were lower only at the beginning of the reproductive season (October-November). The only plausible explanation for this repartition of larvae would be a lower resilience of *C. acutipinnis* spawning population compared to *S. lagocephalus* after cyclonic flood events. A recent study in the same watershed highlighted important movements of adults of both species probably due to the recolonization of upstream habitats after a cyclonic flood by washed down individuals (Lagarde et al., 2015). As *C. acutipinnis* individuals are known to present slightly lesser climbing performances than *S. lagocephalus* (Voegtlé et al., 2002), they would probably take more time to return to their upstream spawning grounds after a cyclonic flood event. The lesser abilities of *C. acutipinnis* to recolonize upstream habitats could explain why the proportion of their larvae was lower than expected at the upstream site, especially after the cyclonic flood occurred.

Is it possible to use larval drift for assessing the biotic integrity of tropical rivers?

Fishes have been used as indicators of the biotic integrity of riverine habitats for years in both temperate (Karr, 1981) and more recently in tropical areas (Mérigoux et al., 2012; Kido, 2013), as they integrate the impact of natural and anthropogenic disturbances on riverine communities. Generally, those indicators are based on estimations of the abundances of adults and juveniles fishes as, in temperate streams, the numbers of adults and juveniles largely depend on the biotic integrity of growing and spawning habitats present in the watersheds. In rivers of tropical islands, the abundances of amphidromous fishes are strongly influenced by the number of juveniles returning in the watershed, and by the biotic integrity of the habitats where they will grow (McDowall and Taylor, 2000). Thus, monitoring adults and juveniles of tropical amphidromous fishes do not directly reflect the biotic integrity of spawning habitat within a watershed because of the marine dispersal phase at the end of which no homing exists (Berrebi et al., 2005). In this context, the abundance of drifting larvae could be a good proxy of the biotic integrity of both growing and spawning habitats since it depends on the biomass of adults and their achievement of reproduction within the watershed. Monitoring larval drift could also represent an interesting complementary approach to classical fish monitoring methods based on electro fishing or visual counts and to adult habitat surveys. Indeed, it integrates the biotic integrity of all habitats up to several kilometers upstream to the sampling site and not only of habitats located in the sampling site. Monitoring larval drift could also allow evaluating species richness (Luton et al. 2005) of rivers, especially as progress in genetic techniques will soon allow efficient larval identification and quantification at the species level (Loh et al., 2014). However, evaluation of species richness would be easier and more efficient using environmental DNA methods (Valentini et al., 2015). Anyway, the challenge will be to obtain unbiased estimation of numbers of larvae for several species in order to use monitoring larval data as complementary indicators of the biotic integrity of riverine habitats.

Before using larval drift for assessing the biotic integrity of riverine habitats, numerous gaps in the knowledge about the biology and ecology of amphidromous species have to be filled. Firstly, knowing how far each species is present upstream to a monitoring site is mandatory. Indeed, the absence of drifting larvae of one species in the samples could simply come from the fact that adults are absent upstream. Secondly, the localization of the growing and spawning habitats of each species (Teichert et al., 2013a; Girard et al., 2014; Teichert et al., 2014a) appears essential. For example Koster et al. (2013) observed that *Prototroctes maraena*, an amphidromous Osmeriformes from Australia, migrated downstream to their spawning grounds located 5 km up from the estuary whereas adults were present up to 30 km upstream during the rest of year. In the case of this species, looking for drifting larvae in upstream reaches would be unsuccessful as growing and spawning habitats of adults were spatially separated. Finally, an estimate of the mortality of drifting larvae (Bell, 2009) would be necessary to evaluate the spatial representativeness of the information obtained at each monitoring site. Indeed, Bell (2009) estimated than more than 99 % of the drifting larvae he caught, hatched in a 5 km section of river upstream from his sampling sites. In this situation, drift samples would be representative of the biotic integrity of riverine habitats located from the sampling site to less than 5 km upstream only. However, Bell's results varied over several orders of magnitude at a given sampling site and between sampling sites. It thus appears important to reevaluate how far upstream the larvae sampled at a given site are coming from in rivers presenting different biotic and abiotic conditions. Once this knowledge is obtained, relationships between larval drift and anthropogenic pressures would be possible and indicators of the biotic integrity of riverine habitats could be developed (Delpech et al., 2010; Kido, 2013). In summary, before using larval drift for assessing the biotic integrity of riverine tropical habitats, future research programs should focus on the spatial distribution of adults of amphidromous fishes, localization of their growing and spawning habitats, and estimates of the mortality of their drifting larvae.

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Fig. 1

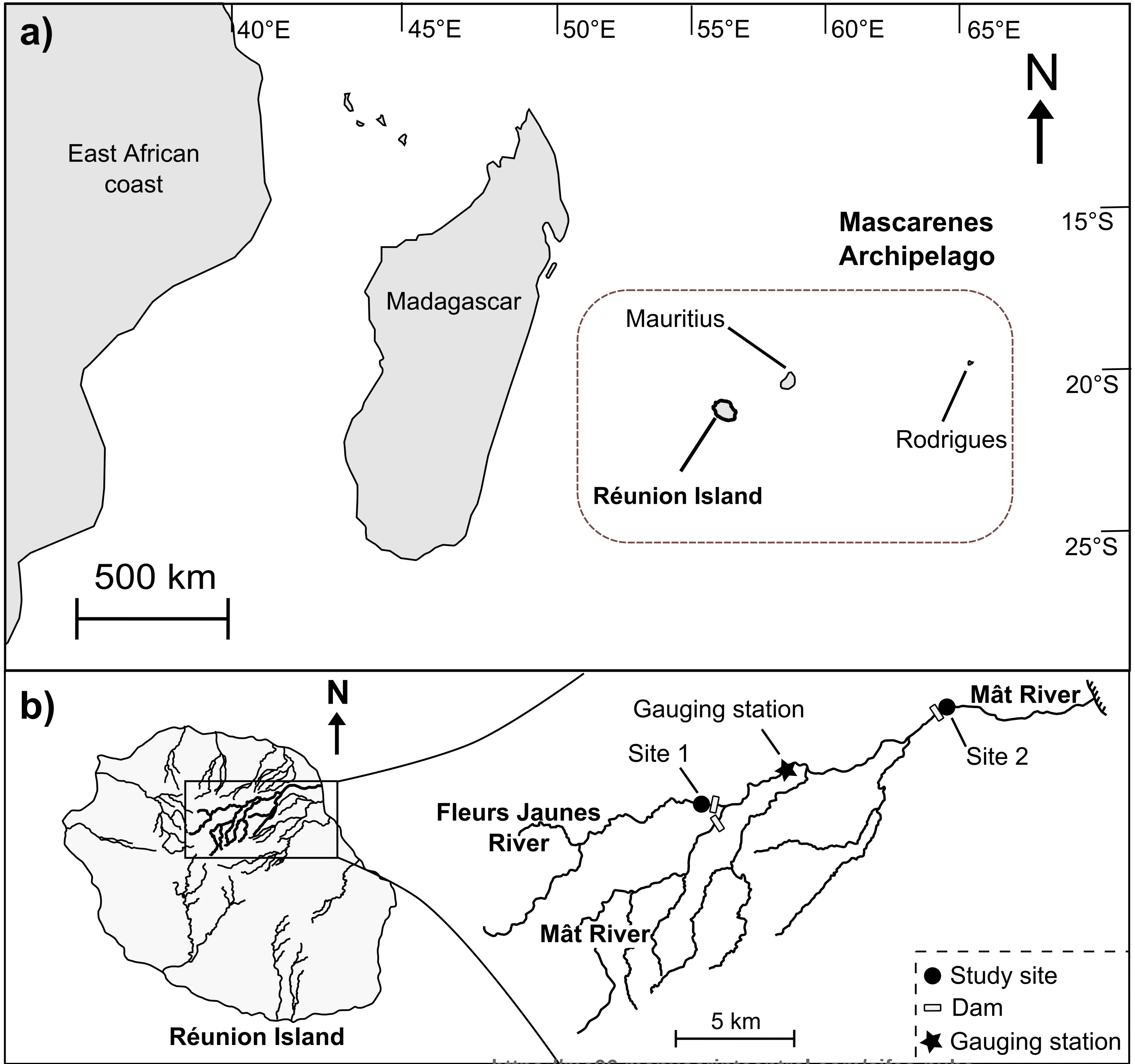
a)
40°E
45°E
50°E
55°E
60°E
65°E
N
East African coast
Madagascar
Mascarenes Archipelago
15°S
20°S
25°S
Mauritius
Rodrigues
Réunion Island
500 km
b)
N
Réunion Island
Gauging station
Site 1
Site 2
Mât River
Fleurs Jaunes River
Mât River
5 km
Study site
Dam
Gauging station

Fig. 2

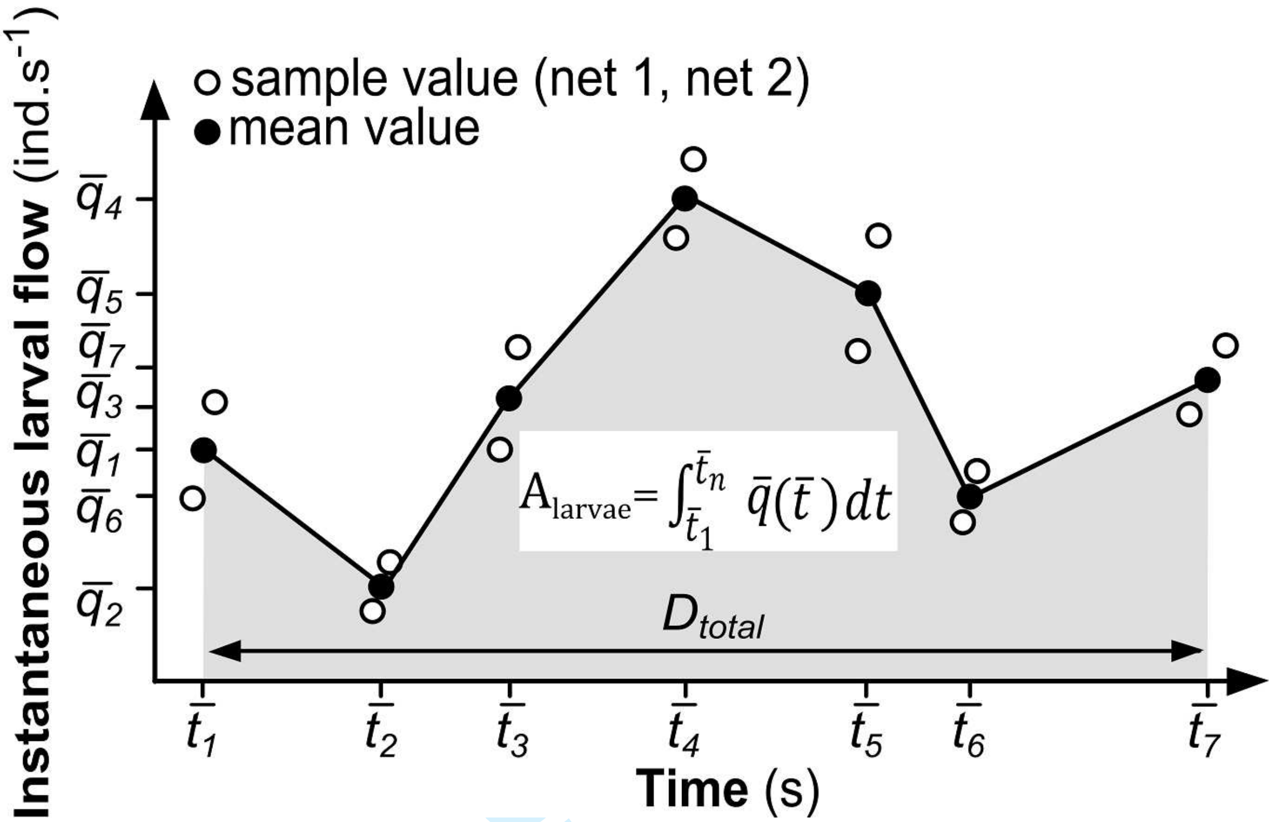
sample value (net 1, net 2)
mean value
Instantaneous larval flow (ind.s^{-1})
$\bar{q}_4$
$\bar{q}_5$
$\bar{q}_7$
$\bar{q}_3$
$\bar{q}_1$
$\bar{q}_6$
$\bar{q}_2$
$A_{larvae} = \int_{\bar{t}_1}^{\bar{t}_n} \bar{q}(\bar{t})\,dt$
D_{total}
$\bar{t}_1$ $\bar{t}_2$ $\bar{t}_3$ $\bar{t}_4$ $\bar{t}_5$ $\bar{t}_6$ $\bar{t}_7$
Time (s)

Fig. 3

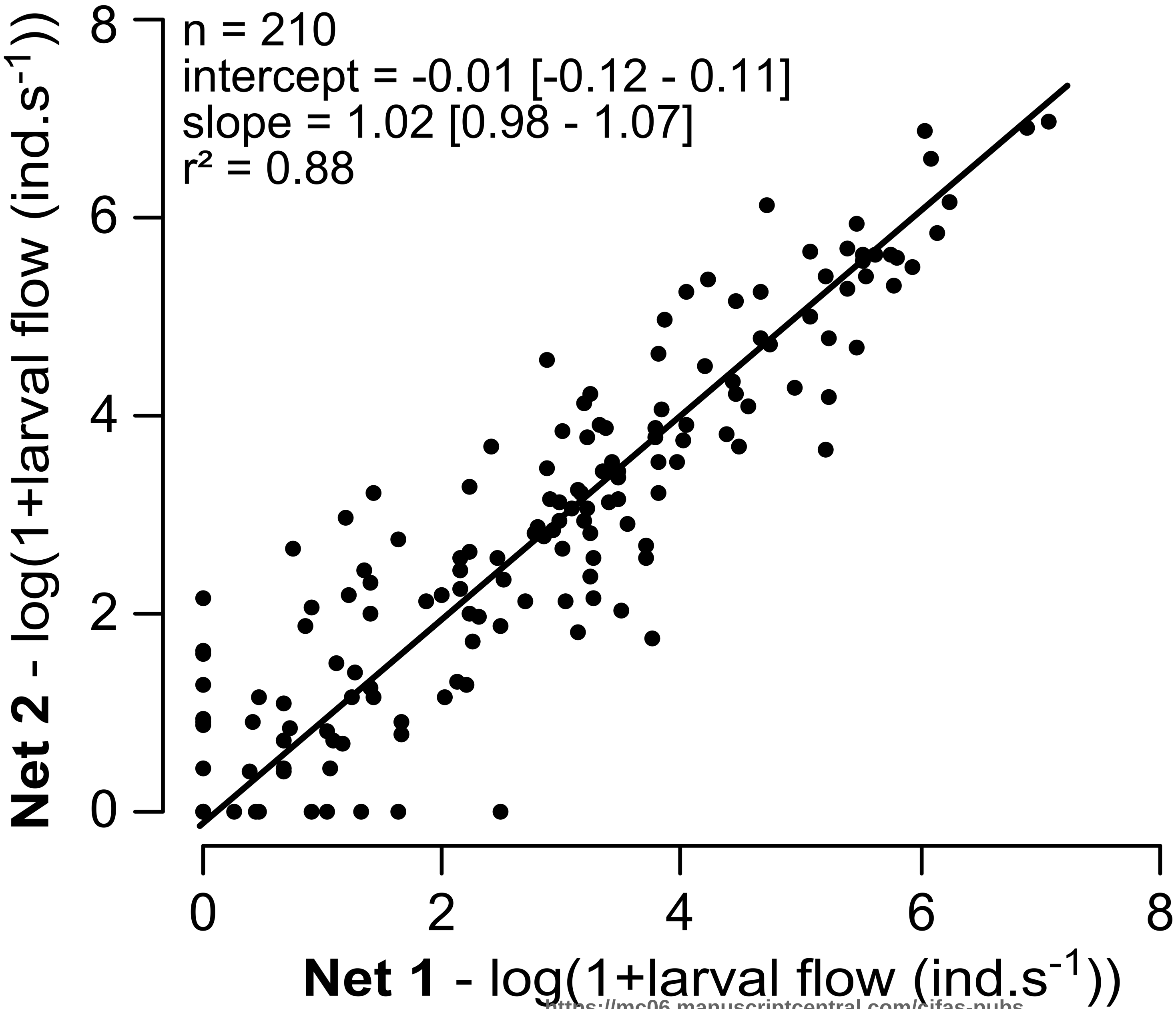
n = 210
intercept = -0.01 [-0.12 - 0.11]
slope = 1.02 [0.98 - 1.07]
r² = 0.88
Net 2 - log(1+larval flow (ind.s-1))
Net 1 - log(1+larval flow (ind.s-1))
0
2
4
6
8

Fig. 4

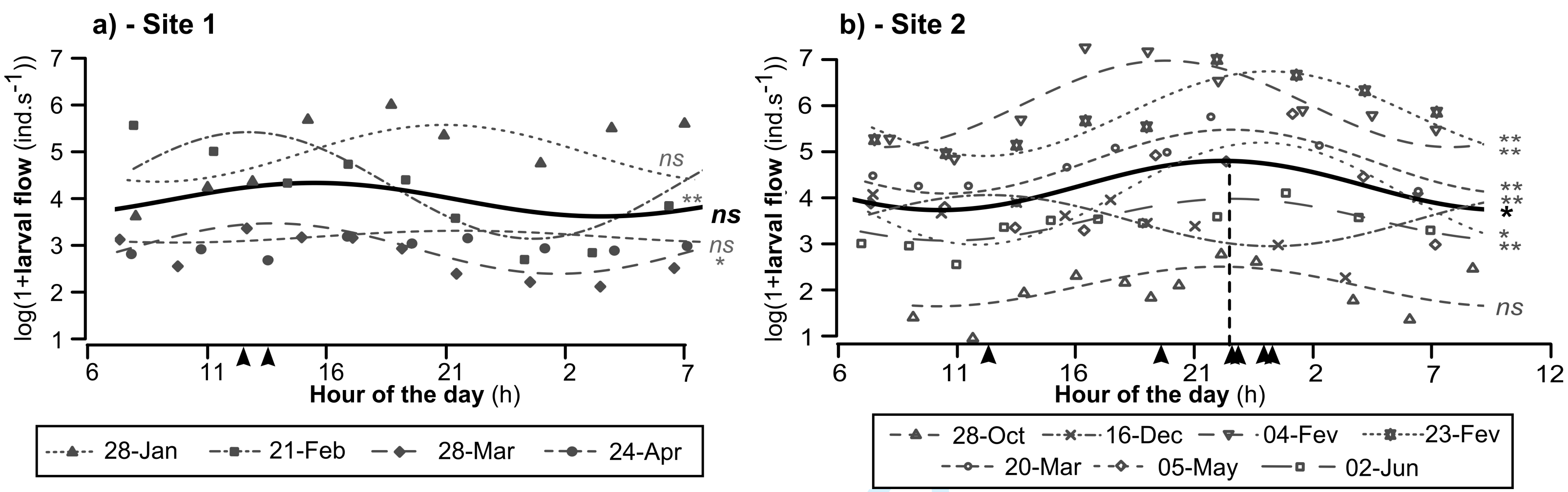
a) - Site 1
b) - Site 2
log(1+larval flow (ind.s^{-1}))
Hour of the day (h)
ns
**
*
28-Jan
21-Feb
28-Mar
24-Apr
28-Oct
16-Dec
04-Fev
23-Fev
20-Mar
05-May
02-Jun

Fig. 5

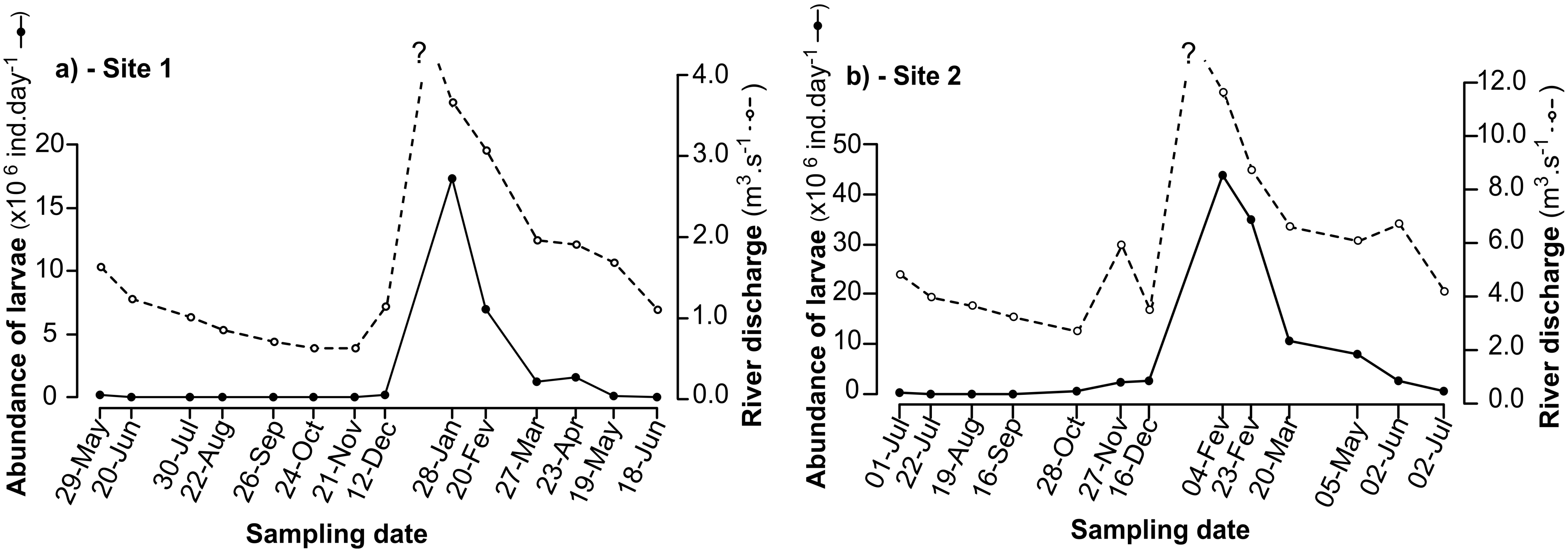
a) - Site 1
Abundance of larvae (x10 6 ind.day-1 —•—)
River discharge (m3.s-1 ·∘·)
0
5
10
15
20
0.0
1.0
2.0
3.0
4.0
?
29-May
20-Jun
30-Jul
22-Aug
26-Sep
24-Oct
21-Nov
12-Dec
28-Jan
20-Fev
27-Mar
23-Apr
19-May
18-Jun
Sampling date
b) - Site 2
Abundance of larvae (x10 6 ind.day-1 —•—)
River discharge (m3.s-1 ·∘·)
0
10
20
30
40
50
0.0
2.0
4.0
6.0
8.0
10.0
12.0
?
01-Jul
22-Jul
19-Aug
16-Sep
28-Oct
27-Nov
16-Dec
04-Fev
23-Fev
20-Mar
05-May
02-Jun
02-Jul
Sampling date

Figure 1. Location of studied sites in the Mât River watershed, Réunion Island, southwestern Indian Ocean.

Figure 2. Schematic representation of the method used for estimating the daily abundance of larvae (A_{larvae}) from instantaneous larval flow (q_{larvae}). $\overline{t}_n$ and $\overline{q}_n$ represent the mean time and mean instantaneous larval flow of the two-nets samples, respectively, and D_{total} the total duration of sampling (i.e. time between the first and the last two-nets samples of the sampling session).

Figure 3. Orthogonal regression of the estimated instantaneous larval flows (log +1) between the fixed net (net 1) and the randomly positioned net (net 2). Data from both sampling sites and all sampling sessions are pooled (n = 210). The values of the intercept and slope are given with 95 % confidence intervals and r^2 is the Pearson's correlation coefficient.

Figure 4. Instantaneous larval flow (log+1) and predictions of the periodic linear mixed models (solid black line) according to the hour of the day for Site 1 (4a) and Site 2 (4b). The vertical dashed line represents the location of the consensual daily peak predicted by the periodic mixed model. The grey dashed lines and associated symbols represent the larval flow (log+1) and the periodic linear model adjusted separately for each sampling session from the 28 January 2014 to the 24 April 2014 (4a) and from the 28 October 2013 to the 02 June 2014 (4b). The significance of each model is indicated by: *ns* (non-significant), * ($P < 0.05$) and ** ($P < 0.01$). The black arrows represent the locations of the individual daily peaks predicted.

Figure 5. Seasonal fluctuation in total daily abundance of larvae (solid line and fill dots) and river discharge measured during the sampling session (dashed line and open dots) for Site 1 (5a) and Site 2 (5b). The discontinuity in the discharge curve (dashed line) gives the approximate date of the cyclonic flood.

Table 1: Summary of the sampling effort and the number of successfully identified individuals of each species for Site 1 and Site 2. [a] sampling stopped for safety reasons (see material and methods). [b] technical issue with one net of the first two-nets samples of the sampling session.

Site	Date	Time of the first two-net sample	N nets	Total filtrated volume (m^3)	Abundance of captured larvae	Mean river discharge ($m^3.s^{-1}$)	N genetically identified	N *S. lagocephalus*	N *C. acutipinnis*
Site 1	29/05/2013	7:50	10	31.9	41	1.6	-	-	-
	20/06/2013	10:20	4	14.4	1	1.2	-	-	-
	30/07/2013	10:32	4	10.5	0	1.0	-	-	-
	22/08/2013	12:56	4	8.0	0	0.8	-	-	-
	26/09/2013	10:19	4	11.2	0	0.7	-	-	-
	24/10/2013	7:35	14	37.6	0	0.6	-	-	-
	21/11/2013	6:45	16	43.1	0	0.6	-	-	-
	12/12/2013	7:10	20	42.9	75	1.1	42	33	9
	28/01/2014	7:48	18	39.9	1842	3.6	50	48	2
	20/02/2014	7:45	18	14.6	436	3.1	47	43	4
	27/03/2014	7:08	20	49.7	371	2.0	47	43	4
	23/04/2014	7:35	18	43.5	420	1.9	56	54	2
	19/05/2014	7:00	18	50.1	36	1.7	33	33	0
	18/06/2014	7:37	14	43.1	0	1.1	-	-	-
Site 2	01/07/2013	12:04	26	83.2	65	4.8	-	-	-
	22/07/2013	11:53	16	45.6	2	3.9	-	-	-
	19/08/2013	10:23	16	44.7	0	3.6	-	-	-
	16/09/2013	10:58	18	55.1	1	3.2	-	-	-
	28/10/2013	9:07	24	58.8	139	2.7	53	46	7
	27/11/2013	9:22	6[a]	17.1	77	5.9	51	29	22
	16/12/2013	7:25	20	49.3	486	3.5	54	54	0
	04/02/2014	8:08	17[b]	16.4	596	11.6	51	47	4
	23/02/2014	7:32	18	17.6	736	8.7	50	49	1
	20/03/2014	7:23	20	42.3	736	6.6	54	53	1
	05/05/2014	7:23	18	43.0	598	6.0	54	46	8
	02/06/2014	7:03	22	71.4	280	6.7	54	42	12
	02/07/2014	7:13	18	55.5	80	4.1	50	48	2

Table 2: Summary of the mixed periodic models for Site 1 and Site 2 with the sampling session as random effect. The significance of fixed effects is based on the Satterthwaite's method.

	Coefficient	Estimate	d.f.	F	*P*
Site 1	β_0	3.69	-	-	-
	β_1	-0.23	5.97	3.66	0.47
	β_2	-0.27	2.99	0.67	0.11
Site 2	β_0	4.14	-	-	-
	β_1	-0.21	5.47	3.94	0.09
	β_2	0.49	5.91	6.14	0.05