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Geographic variation and behavioral evolution in marine plankton: the case of *Mastigias* (Scyphozoa, Rhizostomeae)

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Abstract Although complex behavior in marine zooplankton has been considered strong evidence of adaptation, ethological studies of marine zooplankton generally have not employed either the comparative approach or evolutionary perspective necessary to distinguish adaptation from any alternative. Consequently, the potential for intra-specific variation in the behavior of marine zooplankton has received insufficient attention and conclusions of adaptation remain poorly substantiated. Intra-specific comparison of patterns of migration and behavior for seven populations of golden jellyfish, *Mastigias* (Scyphozoa: Rhizostomeae), inhabiting isolated marine lakes and semi-enclosed lagoon coves in Palau document population specific differences in patterns of horizontal migration, vertical migration, pulse rate, swimming speed, and turning behavior. Evidence was found for symplesiomorphic behaviors, canalization, exaptation, adaptation, and probably once-deleterious traits. Behavioral evolution likely proceeded via, at least, relaxation of selection, trade-offs with morphology, and natural selection effected by predation. Behavioral patterns also may change with ontogeny. Geographic variation in the behavior of marine plankton therefore can be substantial and patterns of evolution complex. Behavioral evolution can rapidly generate coastal biodiversity. Thus, geographic variation in marine plankton is of potential interest to ethologists, evolutionary biologists, biogeographers, and conservation biologists.

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Introduction

In situ studies of marine plankton have demonstrated apparently complex behaviors and strong locomotory abilities in a number of marine zooplankton (Hamner 1985, 1995) which have been considered strong evidence of adaptation (Graham et al. 2001). For example, directional orientation of scyphomedusae into downwelling currents in Langmuir convergences keeps medusae in prey-rich feeding environments (Hamner and Schneider 1986; see also Purcell et al. 2000), whereas circular swimming reduces dispersion when Langmuir cells are not operating (Larson 1992). Sun-compass migration maintains breeding aggregations of medusae in inlets (Hamner et al. 1994), tidally oriented migrations of copepods maintain positions within estuaries (Kimmerer et al. 1998), and wave or wind induced migrations away from shore prevent stranding in jellyfish (Shanks and Graham 1987). Such behavioral characteristics, when displayed by diverse taxa (e.g. Hamner and Schneider 1986; Kimmerer et al. 1998) are strong evidence of selectively beneficial traits (McFall-Ngai 1990). However, widespread behavioral characteristics might also be symplesiomorphic and canalized (Futuyma 1998:441), "spandrels" (sensu Gould and Lewontin 1979) or other characteristics favored by "species hitch-hiking" (Levinton 1988, cited by Futuyma 1998:694), not selected against, or mildly deleterious (Foster

1999). In the absence of additional information, such as a behavioral "fossil record" or a comprehensive phylogeny, the origins and evolution of behaviors are difficult to ascertain. The difficulties are potentially greater the more distant are the taxa being compared and interpretation obfuscated when typological approaches ignore intra-specific variation (Foster 1999). In contrast, the origins and evolution of behavior often can be clarified by comparisons between populations within a species (Foster et al. 1996).

Inter-population variation in behavior has been documented in numerous terrestrial, freshwater, and marine vertebrates (e.g. Endler 1995; Foster et al. 1996; Slabbekoorn and Smith 2002) and in terrestrial and freshwater invertebrates (e.g. Hairston 1980; Rhode et al. 2001; Burks et al. 2002; Shephard et al. 2002). There have been several studies comparing inter-population variation in the behavior of benthic marine invertebrates (e.g. Scapini et al. 1999; Saigusa 2001) but only one species of marine zooplankton has been investigated (Hamner and Hauri 1981). Variation among populations of *Stomolophus meleagris* (Shanks and Graham 1987) in reality probably constituted variation among different aggregations, whereas variation among populations of *Aurelia aurita* (Arai 1997) most likely involved variation among cryptic species (Dawson and Martin 2001). Nonetheless, few studies have explored the evolution of intra-specific geographic variation in behavior (Foster 1999).

In a comparison of golden jellyfish, *Mastigias* sp., populations inhabiting landlocked marine lakes, Palau, we found remarkable diel horizontal and vertical migrations that differed in each of three lakes (Hamner and Hauri 1981). Like the behaviors attributed to different species noted above, intra-specific variations in behavior were considered adaptive. For example, daily horizontal migrations coupled with shadow-avoidance behavior maintained the medusae and their photosymbiotic zooxanthellae in direct sunlight (Hamner and Hauri 1981) whereas nightly vertical migrations to nutrient-rich deeper waters would fertilize the zooxanthellae, providing nutrients needed for daily photosynthesis (Hamner et al. 1982; Muscatine and Marian 1982). However, behavioral differences observed between these three populations of medusae added complexity rather than clarification and led to less satisfactory adaptive explanations (Hamner and Hauri 1981). A serendipitous encounter with congeneric *Mastigias papua* off Townsville, Australia, suggested that oriented migrations using the sun as a proximal cue might be widespread in the genus, but provided neither "fossil" nor phylogenetic insight to facilitate interpretation of these different behavior patterns.

Here, we investigate further the behavioral evolution of *Mastigias* medusae in the marine lakes. We present data describing the behavior of eight populations: the three originally studied by Hamner and Hauri (1981), three occurring in sheltered coves in Palau, and two in additional lakes (Hamner and Hamner 1998). Of exceptional importance to our analysis herein, the cove populations of *Mastigias* are the closest known relatives of the marine lake *Mastigias* (Hamner and Hauri 1981; Dawson, submitted). It is highly likely that the cove populations not only gave rise to the marine lake populations but that they also are similar to the ancestral form (Dawson, submitted; see for analogy Bell and Foster 1994). Moreover, one of the two additional lake populations appears to be evolutionarily young, representing a stage intermediate between lagoon and older lake populations (Dawson, submitted). Thus, this study presents an important historical perspective that has been lacking from prior studies of the behavior of marine zooplankton.

Materials and methods

Data collection

Mastigias were studied in eight locations (Fig. 1): Big Jellyfish Lake (BJLK; November 1996–May 1997), Goby Lake (GLK; May 1996–May 1997), and Big Jellyfish Cove (BJCK; April 1996) on the island of Koror, Ongeim'l Tketau [OTM (the "Jellyfish Lake" of Hamner and Hauri 1981); January–April 1997] and Clearwater Lake (CLM; September–October 1998) on the island of Mecherchar, Risong Cove on Auluptagel (RCA; February–May 1997, August–September 2001), Ongael Lake on Ongael (OLO; October–November 1998), and Ngerchaol Cove on Ngerchaol (NCN; July 1999–August 2001). In each location, vertical water column structure (dissolved oxygen, salinity, temperature) was measured using an YSI 85 meter, Secchi disk depths were recorded, weather patterns noted (and supplemented with data from the weather station at Koror), and the distribution of shadow-lines was mapped for each hour of daylight. Seven aspects of the behavior of *Mastigias* were measured.

1. The horizontal distribution of *Mastigias* was assessed four times during the day (approximately early-morning, mid-morning, noon, and late-afternoon) in all lakes and once at night (under moonlight) in BJLK, GLK, and OTM, but not in CLM due to the presence of crocodiles, OLO due to bad weather, nor the coves due to densities of *Mastigias* that were too low to sample effectively using the methods available. In all marine lakes, bar OLO, a plankton net (0.11 m^2, 1-mm^2 mesh) was hauled vertically from 1 m below the chemocline or from the lake bottom

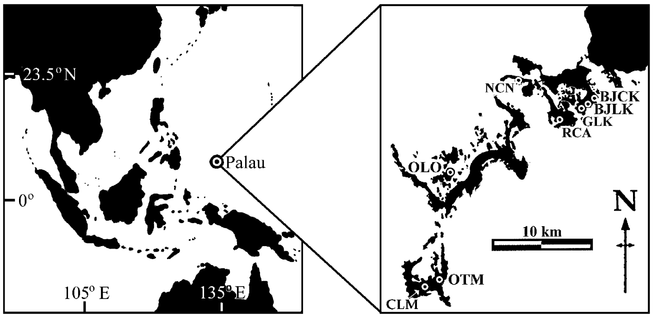


Fig. 1 Maps showing the position of Palau in the western Pacific Ocean and the locations of the coves (*BJCK, NCN, RCA*) and marine lakes (*BJLK, CLM, GLK, OLO, OTM*) in Palau in which *Mastigias* were studied. *BJCK* Big Jellyfish Cove, Koror; *NCN* Ngerchaol Cove, Ngerchaol; *RCA* Risong Cove, Auluptagel; *BJLK* Big Jellyfish Lake, Koror; *CLM* Clear Lake, Mecherchar; *GLK* Goby Lake, Koror; *OLO* Ongael Lake, Ongael; *OTM* Ongeim'l Tketau, Mecherchar (aka. Jellyfish Lake, Hamner and Hauri 1981)

(whichever was shallower) to the lake surface at a rate of approximately 0.3 ms^{-1} at 14 or 15 sites (depending on the lake). The contents of each haul were individually bagged and labeled before the next haul began. The frequency of small [$\leq$ 4 cm bell diameter (bd)], medium (4 cm < bd $\leq$ 8 cm), large (8 cm < bd $\leq$ 12 cm), and, if present, very large (> 12 cm) medusae was counted in all hauls upon completion of the final haul. In OLO, vertical net hauls were ineffective due to a lower density of *Mastigias*, so an open-ended polyurethane cylinder 1.5 m diameter and 0.5 m deep was dropped vertically into the water. Medusae deeper than 0.5 m were counted immediately before they swam out of, or others swam into, the sampling area, and subsequently all medusae trapped within the device (i.e. shallower than 0.5 m) were counted. All lakes were sampled as quickly as possible, within 30–75 min (mostly between 45–60 min) depending on the size of the lake, providing relatively instantaneous "snapshots" of *Mastigias* distributions. In coves, where even lower densities of *Mastigias* precluded sampling by either method used in the lakes, medusae near the surface ($\leq$ 2–3 m deep) were counted from a vantage point on a small boat traversing a predetermined course. Sampling across 14 sectors (covering the entire area of each cove) was completed within 10 min.

2. Instantaneous swimming direction (ISD; i.e. compass bearing) of *Mastigias* was measured to the nearest 5° interval at three times of day (bar CLM, 2 times). Medusae were selected haphazardly by a snorkeller finning five strokes with closed eyes, then choosing the first medusa of the next size-class (small, medium, large, or very large, chosen on a rotational basis) seen upon opening their eyes.
3. The vertical distribution of *Mastigias* medusae was assessed at noon and at night [except CLM, BJCK at night for the reasons explained in 1 above]. In all lakes, a weighted vertical down-line was hung in the middle of each lake. At 2 m depth intervals a diver using SCUBA swam horizontally for 10 m holding in front of them a plankton net (0.11 m^2, 1-mm^2 mesh) and, at the end of the swim, transferred the caught medusae into individually labeled, draw-string mesh bags. When all depths were sampled, divers returned to the surface and counted the number of medusae in each size class at each depth. In CLM and OLO, the daytime vertical distribution of *Mastigias* medusae was assessed by trawling 10 m with a weighted plankton net (0.11 m^2, 1-mm^2 mesh) hung on a graduated down-line deployed from a surface raft. The net was weighted such that it could be deployed, run, and recovered with the mouth oriented vertically, so medusae were caught in the net only while it was being towed horizontally. CLM was sampled at 2 m and OLO at 1 m depth intervals. In coves, sampling with nets generally was precluded by the low density of *Mastigias* so the number of medusae counted at or near the surface during assessment of horizontal distributions was used as an indicator of the vertical distribution of medusae. On one occasion, an unusually high density of *Mastigias* medusae in RCA permitted sampling by divers with a net as described above except over two 2 min timed transects rather than one 10 m measured transect.
4. Swimming behavior was studied by following haphazardly selected medusae of all sizes for several minutes and noting, at 10-s intervals, their ISD.
5. The response of *Mastigias* to shadows was documented by haphazardly selecting medusae of all sizes and documenting their responses to either the natural shadow line at the edge of lakes and coves or to the shadow cast by a raft in the middle of lakes. The position of turns and number of pulses made by medusae relative to the shadowline were recorded.
6. Pulse rates of *Mastigias* were measured using haphazardly selected medusae of all sizes. The time it took medusae to complete 60 pulses in situ was recorded. In addition, the pulse rates of *Mastigias* from CLM were measured in the laboratory at three different temperatures.
7. Swimming speed was measured using haphazardly selected medusae of all sizes. Water adjacent to the oral arms of a medusa was marked with fluoroscein dye at time zero and again 0.5 m later. The time taken to traverse the 0.5 m was recorded, if the medusa had swum in a straight line for the entire distance, and swimming speed (ms^{-1}) calculated.

Two additional sets of measurements were made. First, the density of the anemone *Entacmaea medusivora* $\leq$ 5 m deep was measured semi-quantitatively in OTM. Quadrats of 0.25 m^2 were selected around the circumference of OTM to reflect, as best possible, the range of densities of anemones. Second, the frequency with which *Mastigias* bumped into shaded or illuminated areas of benthos $\leq$ 5 m deep in BJLK was recorded on four occasions. These data were used to estimate historical levels of mortality of *Mastigias* in OTM attributable to *E. medusivora*.

Data analysis

Hamner and Hauri (1981) demonstrated different patterns of migration by *Mastigias* under sunny and overcast conditions. As such, all data were classified according to the predominant weather during measurement. Data classified similarly by weather (within locations and time-of-day) were grouped for analysis while data classified dissimilarly (by weather, location, or time-of-day) were analyzed separately and then compared. Data analyses were completed using SYSTAT v. 6.0 for Windows 3.1 or equations written in Microsoft Excel 98 for PPC. Data were tested for conformity to the assumptions [e.g. normality (Lilliefors test) and homogeneity of variances (*F*-test or Cochran's test; Winer et al. 1991)] of the relevant statistical procedures (e.g. ANOVA, correlation). When assumptions were not met, data were transformed to meet the assumptions, an alternative approach employed (e.g. multiple *t*-tests, assuming heterogeneous variances), or ANOVA was performed and the results interpreted conservatively (see Underwood 1997). When appropriate, sequential Bonferroni corrections were applied (Rice 1989):

1. Horizontal distribution. In each cove and lake, all vertical hauls were attributed to one of up to five sectors along an east-west axis (see maps in Fig. 2). The number of medusae caught (i.e. "observed") was summed within each sector and across all sectors. The total across all sectors was used to calculate the number of medusae expected in each sector if medusae were distributed randomly (i.e. uniformly) by multiplying the total number of medusae "observed" by the proportion of the hauls that were made in each sector. The observed distribution was compared to the expected distribution (the equivalent of a null hypothesis of no migration) using the χ^2 goodness-of-fit test.
2. Instantaneous swimming direction (ISD). For each sample, the χ^2 goodness-of-fit test was used to compare the distribution of observed bearings (ϑ_i) grouped into 30° classes, with an uniform distribution representing the null hypothesis of no directional migration. The null hypothesis was estimated according to the equation $m = n/k$, where m is the expected number of observations per 30° class, n is the sample size, and k is the number of classes (in this case 12; Fisher 1993). In a minority of cases, observed bearings were grouped into 60° classes ($k = 6$) to meet the requirement $m \geq 2$ (Fisher 1993). The mean ISD (mISD) and its variance were calculated (Fisher 1993).
3. Vertical distribution. Using the χ^2 goodness-of-fit test, the observed vertical distribution of medusae was compared against a null (uniform) distribution calculated by dividing the total number of medusae caught by the number of depth intervals. The weighted mean depth (WMD) of observed vertical distributions was calculated following Pearre (1973).
4. Swimming behavior. To control for any effects of size, the size distributions of medusae sampled from different populations were compared using ANOVA. Statistically similar samples were compared further on the basis of their swimming behavior, characterized as the frequency and magnitude of turns (i.e. the difference between sequential bearings measured in the field) made by medusae, using multiple pairwise *t*-tests due to heteroschedastic data.

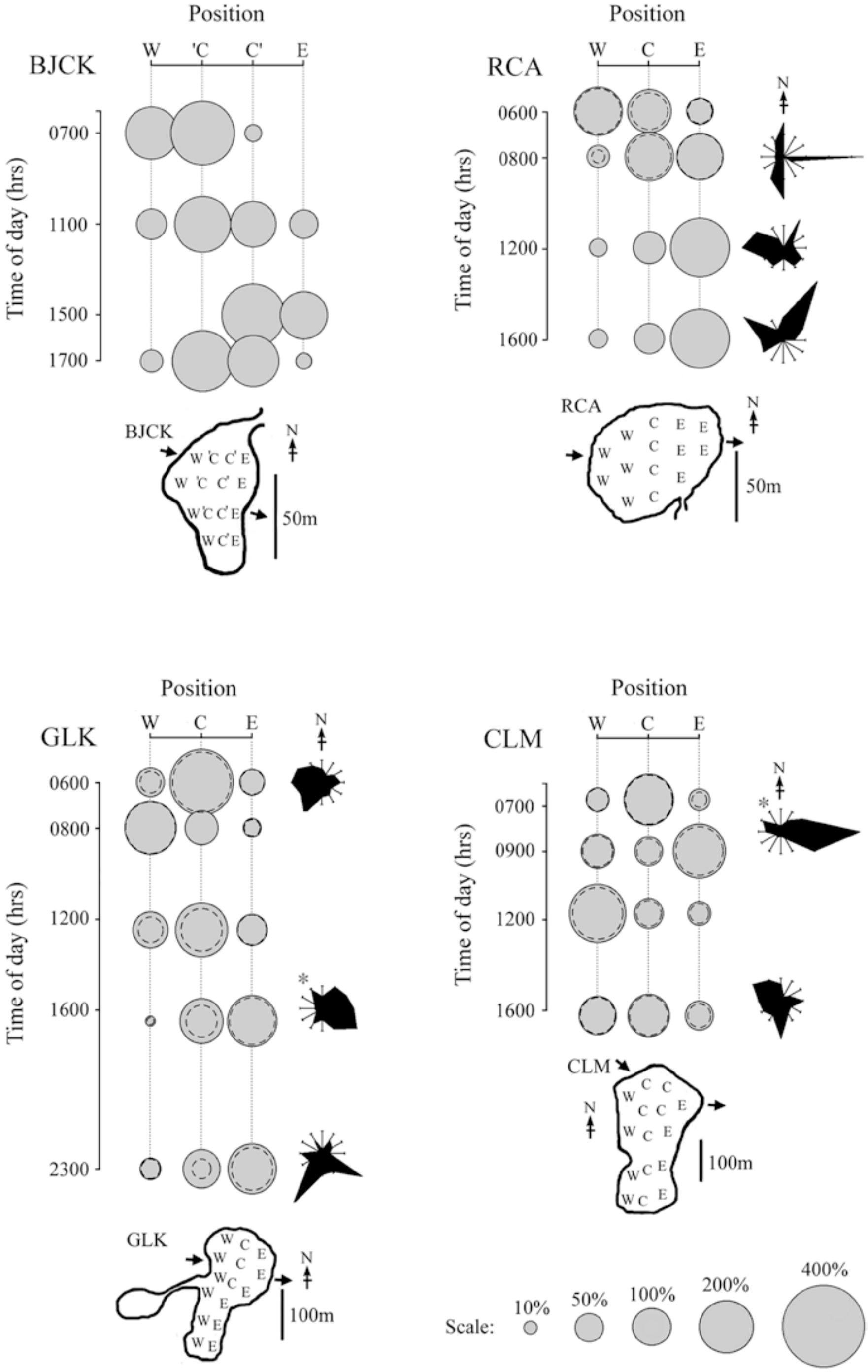
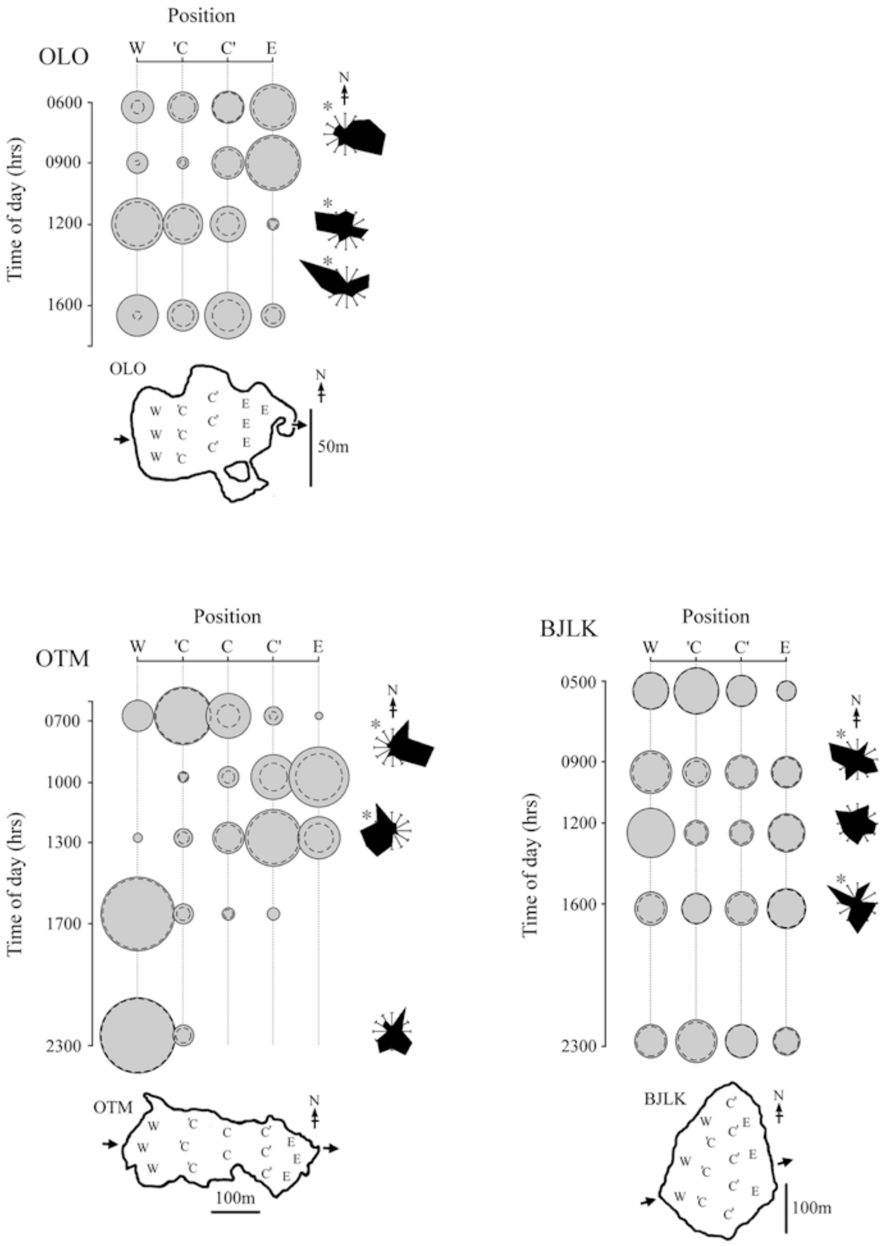


Fig. 2 Horizontal distribution along west-east axes and instantaneous swimming directions of *Mastigias*. Horizontal distribution (*bubble plot*): mean number of medusae (*solid circle*) in each sector as a percentage of the number expected were they uniformly distributed (i.e. a value of 100% means the observed value was equal to the expected value). *No circle* indicates that medusae were absent from, or present at very low densities in, that sector. *Dashed circles* show mean minus 1 SE. All distributions are significantly non-uniform (χ^2 goodness-of-fit test, $P < 0.005$) after sequential Bonferroni correction across times within each lake (see Electronic supplementary material SD1-I). Instantaneous swimming direction (ISD; *rose diagram*): the length of bars, which occur at 30° intervals starting at 000°, are scaled to 0.1 of the sample size. ISDs marked with an asterisk differed significantly from random ($P \leq 0.01$; see Electronic supplementary material SD1-II). *Sketches below bubble plots* indicate the sample sites within each basin. *Arrows* indicate the positions at which sunlight first and last illuminated the water and the direction in which sunlight, and shadows, tracked across each location. See Fig. 1 for list of site acronyms

5. Shadow response data were multifarious and poorly suited to statistical comparison. Samples are described statistically where possible and compared preliminarily and qualitatively.
6. Pulse rates, $\log_{10}$ transformed, were related to $\log_{10}$ bell diameter using ordinary least-squares regression. The regressions could not be compared using analysis of covariance due to significantly different slopes (see "Results"). Instead, pulse rates were compared between lakes using the two best sampled size categories (38–45 mm and 115–135 mm) corresponding, approximately, to the smallest and largest medusae. The size distributions of medusae sampled from different populations were compared within each size category using ANOVA, to ensure there were no statistically significant differences in size that might confound interpretation of data describing variation in pulse rates. The pulse rates of medusae from each lake were compared, within size classes, using ANOVA and multiple post-hoc pairwise comparisons completed using the Tukey test. Pulse rates of medusae in different temperature regimes were compared using ANOVA and multiple pairwise comparisons completed using the Bonferroni post-hoc test.
7. Swimming speeds were related to bell diameter using ordinary least-squares regression. Subsequently, swimming speeds were compared between lakes using ANOVA because the slopes of all regressions were statistically indistinguishable from zero. Multiple post-hoc pairwise comparisons were completed using the

Fig. 2 (Contd.)



Tukey test. Correlations between swimming speeds and pulse rates were explored in the smallest and largest size classes of medusae (38–45 mm and 115–135 mm).

Results

The coves, BJCK and RCA, and one lake, OLO, were well-mixed (“holomictic”), being almost isothermal and isohaline with depth. Although concentrations of dissolved oxygen were variable with depth, the water columns were generally well oxygenated throughout and never anoxic (Fig. 3). The cove NCN also was holomictic, with mean [±1 SE; $n = 30$ (2 days, 15 depths)] temperature $29.6 \pm 0.2^{\circ}$C, salinity 34.5 ± 0.11‰, and dissolved oxygen 5.9 ± 0.14 mgl^{-1}. In contrast, the water columns of the deeper marine lakes, GLK, CLM, OTM, and BJLK, were all highly stratified by salinity or temperature (Fig. 3). These meromictic lakes were characterized by dissolved oxygen levels that decreased rapidly with increasing depth, reaching zero at a chemocline often marked by dense accumulations of purple-sulfur bacteria. Water clarity, as indicated by Secchi disk depth (Fig. 3), was greatest in Risong Cove (RCA) and less in all marine lakes. Water clarity in the lakes was correlated with the depth of both chemocline ($r = 0.996$, $n = 3$) and lake ($r = 0.997$, $n = 4$).

The morning’s retreating shadows and the afternoon’s advancing shadows tracked from west to east across each location (Fig. 2). The timing of first light, last light, and the progression of shadows varied between locations (e.g. BJCK 0800–1530 hours and OTM 0600–1730 hours) due to differences in the time of year and basin topography. Normalizing for time of year, by calculating illumination as a percentage of possible

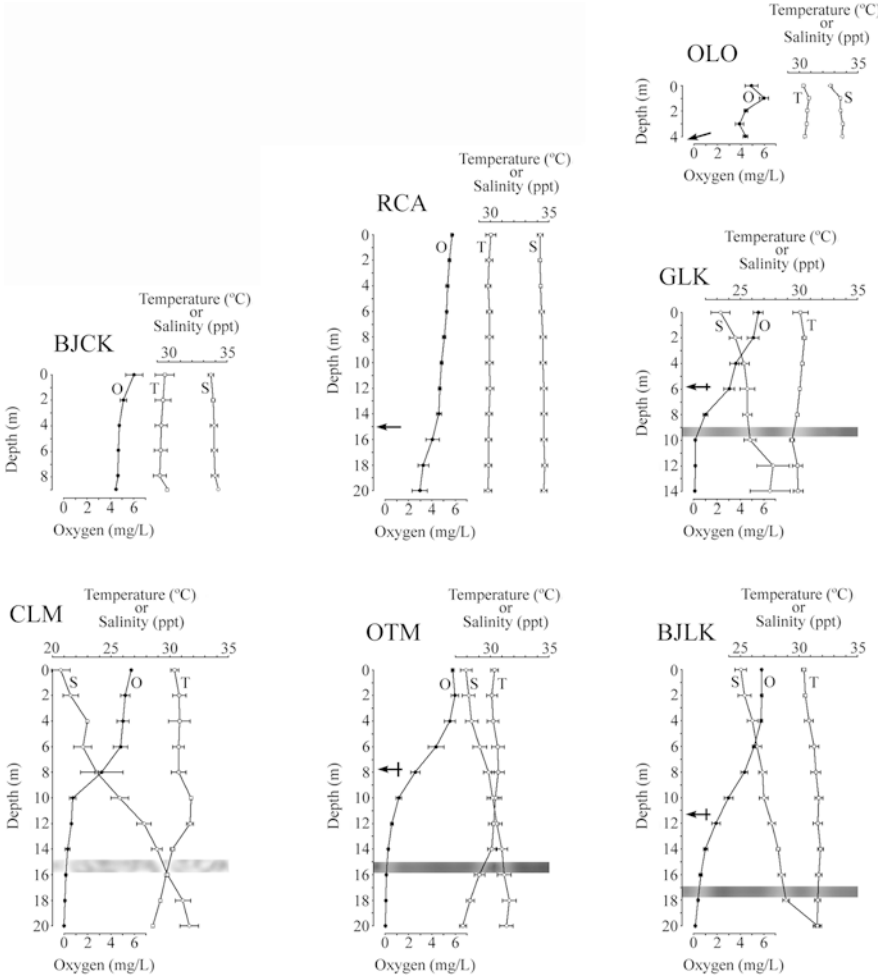


Fig. 3 Vertical water column structure of study locations. *BJCK* 2 profiles in July 1999 and August 2001 (Secchi disk depth not measured); *RCA* 4 profiles between July 1999 and February 2001 (2 Secchi measurements only); *OLO* 3 profiles between October 1997 and June 2001 (2 Secchi measurements only, both ≥depth of the lake); *GLK* 5 profiles between October 1978 and February 2001; *CLM* 4 profiles between October 1979 and October 1998 (Secchi disk depth not measured); *OTM* 6 profiles between April 1979 and March 2001; *BJLK* 8 profiles between December 1982 and December 2000. Profiles show mean (± *1 SE*) dissolved oxygen (*O*), temperature (*T*), salinity (*S*). Mean Secchi disk depth (± *1 SE*) is indicated by an *arrow* against the ordinal axis. The position of the chemocline, if present, is shown by *shading*. *Lighter shading* indicates a possibly less well developed chemocline as indicated by greater dispersion of red sulfur bacteria (Hamner and Hamner 1998). Data between 1978 and 1982 were extracted from Hamner and Hamner (1998)

daylight (11.9 h in October to 12.5 h in May), small basins surrounded by high ridges were illuminated considerably less than large basins surrounded by low ridges: BJCK 61%, GLK 65%, CLM 71%, RCA 72%, BJLK 76%, OLO 80%, OTM 94%.

1. Horizontal distribution (Fig. 2). *Mastigias* were non-uniformly distributed in all coves and lakes at all times (χ^2 test, $p \leq 0.0013$; GLK $n = 2$, OTM $n = 4$, all other sites $n = 3$). Distributions were predominantly unimodal and highly skewed, although several showed a weak second peak in density (CLM 0900 hours; OLO 0600, 0900, 1600 hours; BJLK 0900, 1200 hours). *Mastigias* in the coves occurred at higher densities than expected in westerly sectors and lower densities than expected in easterly sectors during the early morning (BJCK 0700 hours; RCA 0600 hours). Distributions subsequently shifted eastward such that *Mastigias* in BJCK and RCA occurred at lower densities than expected in westerly sectors and higher densities than expected in easterly sectors during mid-afternoon (e.g. BJCK 1500 hours; RCA 1600 hours). The shift in distribution occurred earlier in RCA than in BJCK. By late afternoon (1700 hours) a shift toward central sectors was obvious in the distribution of *Mastigias* in BJCK. Horizontal distributions of *Mastigias* in marine lakes (Fig. 2) shared some features with those observed in coves although, as in the coves, the timing and magnitude of aggregations varied between lakes. *Mastigias* generally occurred at higher densities than expected in westerly sectors and lower densities than expected in easterly sectors during the early morning (e.g. BJLK 0500–0900 hours, OTM 0700 hours, GLK 0800 hours), although *Mastigias* occurred mostly in the central sector in CLM (0700 hours; see also GLK 0600 hours) and toward eastern or western extremes in OLO (0600 hours; dependent on the day). In all lakes, the distribution of *Mastigias* subsequently shifted toward the east. The eastward shift occurred throughout the day in GLK and BJLK producing the highest densities in the most easterly sectors of both lakes by 1600 hours (Fig. 2). In contrast, the eastward shift occurred in a few hours in CLM, OLO, and OTM—peak easterly distributions were evident by mid-morning (CLM and OLO 0900 hours, OTM 1000 hours)—and were followed quickly by shifts westward. By noon, *Mastigias* in CLM and OLO were distributed principally in western sectors of the lakes, and the highest density of *Mastigias* in OTM also had shifted westward, although it did not reach

its most westerly extreme until late afternoon (1700 hours), by which time the distributions of *Mastigias* in CLM and OLO had become more evenly (but not uniformly) distributed. Horizontal distributions at night-time, measured only in three lakes, generally reflected distributions during the late afternoon. Thus, *Mastigias* in GLK were biased toward the east, *Mastigias* in OTM were biased toward the west, and *Mastigias* in BJLK were relatively evenly (but not uniformly) distributed (2300 hours). However, *Mastigias* were clearly redistributed by morning, often more centrally in lakes (but see OLO) and more westerly in coves. In at least two lakes, ontogeny also appeared to influence migration, resulting in bimodal distributions. In BJLK (1200 hours), "small" medusae were encountered principally in the west (mean% ± SE, 57% ± 0.7) but rarely in the east (10% ± 0.8), while "large" medusae were encountered principally in the east (60% ± 7.9) and more rarely in the west (22% ± 3.8); "medium" medusae were encountered similarly in the east and west (38% ± 5.4 and 32% ± 0.2, respectively). In CLM (0900 hours), "small" medusae were encountered principally in the east (mean% ± SE, 67% ± 8.0) but rarely in the west (10% ± 2.1), while "large" medusae were encountered principally in the west (54% ± 1.6) and more rarely in the east (22% ± 2.4); "medium" medusae were encountered more frequently in the east than west (48% ± 11.5 and 20% ± 3.1, respectively). In both lakes, "small" medusae had greatest influence on the overall distribution because they were the most numerous (57% of population in BJLK; 46% of population in CLM). The apparently bimodal distributions in OLO were attributable to differences between days, not differences between medusae.

2. Instantaneous swimming directions generally were consistent with changes in the horizontal distributions of *Mastigias* (Fig. 2). Mean ISDs were easterly ($83^\circ \leq \mathrm{mISD} \leq 108^\circ$) in all locations (bar BJLK) during the periods in which peaks in the distribution of *Mastigias* shifted from western to eastern sectors (OLO 0730 hours, GLK 1600 hours, CLM 0800 hours, OTM 0800 hours; see also RCA 0800 hours). Reciprocally, mean ISDs were westerly ($256^\circ \leq \mathrm{mISD} \leq 323^\circ$) during the periods in which peaks in the distribution of *Mastigias* predominantly shifted from eastern to western sectors (OLO 1200 hours, OTM 1300 hours; see also GLK 0600 hours,). However, ISDs were never unidirectional, rose diagrams being characterized by between two (e.g. CLM 0800 hours, OLO 1600 hours) and four peaks (e.g. BJLK 0900, 1600 hours). In general, daytime variance in ISDs was smaller in locations in which shifts in the daytime distribution of *Mastigias* were larger (e.g. OTM variance = 0.52–0.60 cf. BJLK variance = 0.87–0.97).
3. Vertical distribution. *Mastigias* were non-randomly distributed in all locations studied during the day (χ^2test, $P < 0.01$; Fig. 4). *Mastigias* also were non-randomly distributed in all lakes (χ^2 test, $P < 0.01$) but not RCA at night ($P = 0.08$; Fig. 4). *Mastigias* were distributed shallower during the day than during the night, as indicated by both maximum depth and WMD. The vertical distribution of *Mastigias* also varied during daylight. *Mastigias* in marine lakes were distributed shallower when the sun was obscured by clouds than during sunny spells (Fig. 5). In contrast, *Mastigias* in RCA (Fig. 4) and NCN (Fig. 5) were mostly found near the surface even in unameliorated noon-day sun and, as such, could not be distributed shallower during overcast weather.

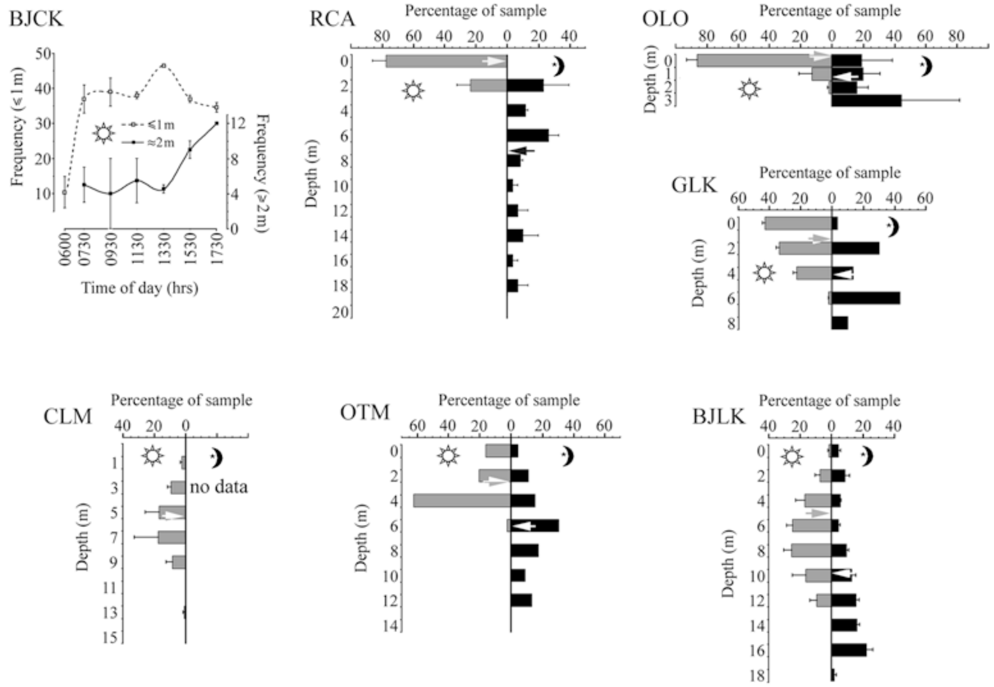


Fig. 4 Vertical distribution of *Mastigias* during daylight (noon; *light bars with sun icon*) and at night [2200 hours, except *OLO* (0500 hours); *black bars with moonstar icon*]. *Solid bars* show the mean percentage of medusae present at each depth and *error bars* indicate 1 SE. *Arrows* indicate weighted mean depth (*WMD*). All distributions were significantly different from uniform (χ^2 goodness-of-fit test, $p < 0.01$) except Risong Cove at night ($P = 0.08$) after sequential Bonferroni correction across times within lakes (see Electronic supplementary material SD2-I). See Fig. 1 for list of site acronyms

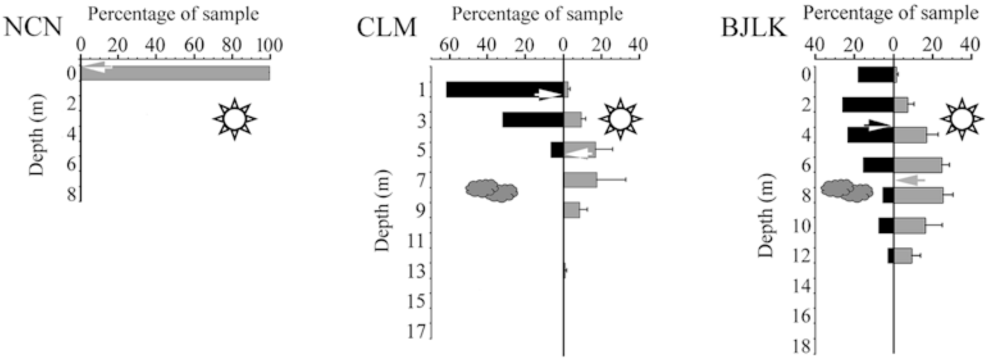


Fig. 5 Vertical distribution of *Mastigias* during sunny (noon; *light bars with sun icon*) and overcast (noon; *black bars with cloud icon*). *Solid bars* show the mean percentage of medusae present at each depth and *error bars* indicate 1 SE. *Arrows* indicate weighted mean depth (*WMD*). All distributions were significantly different from uniform (χ^2 goodness-of-fit test, $P < 0.01$) after sequential Bonferroni correction across conditions within lakes (see Electronic supplementary material SD2-II). See Fig. 1 for list of site acronyms

However, on two occasions, *Mastigias* in RCA were distributed deeper at noon when surface waters were roughened and daylight severely reduced during heavy rainstorms.

4. Swimming behavior (Table 1). The amplitude and frequency of turns differed between the four locations studied. There was no significant difference in turning behavior between BJLK and GLK medusae, but these medusae did make significantly larger turns, on average, than those in RCA and OTM (*t*-test, $P < 0.001$). Turns by *Mastigias* were least frequent and smallest in OTM. Within lakes, swimming behavior varied with weather conditions and time of day. For example, in BJLK, turns were of significantly greater mean magnitude at noon in sunny weather ($n_{\mathrm{turns}} = 208$) than at noon in cloudy weather ($n = 55$; mean ± SE: 52.7° ± 6.54° vs 32.6° ± 2.01°; $t_{65} = 2.94$, $P = 0.005$). Also in BJLK, turns were of significantly greater mean magnitude at noon in cloudy weather than during cloudy mid-afternoons ($n = 66$; 32.6° ± 2.01° vs 18.3° ± 2.01°; $t_{198} = 5.03$, $P < 0.001$). A similar pattern was seen in OLO where turns were of greatest magnitude during noon in the sun ($n = 18$; 42.2° ± 9.3°) and less during overcast mornings ($n = 49$; 27.1° ± 3.3°) or in the shade in the late afternoon ($n = 42$; 22.5° ± 3.4°). Data for other lakes are not available.
5. Ninety-six percent of medusae responded to shadows ($n = 206$; Table 2). Forty-two percent of medusae avoided shadows at the lake-side by swimming parallel to, or turning before they crossed, the shadowline. The percentage avoiding the less intense shadow cast by the raft was only 5%. Medusae that crossed either shadowline (lakeside or raft) generally responded quickly and unambiguously to the shade. Medusae turned between 30° and 180°, apparently depending on whether they approached the shadow obliquely or orthogonally, and all but a few exited the shade near to their point of entry in less (usually much less) than one minute.
6. Pulse rates varied with bell diameter and between locations (Table 3). Comparison of log-log transformed data indicated the relationship between pulse-rate and bell diameter was lake specific (ANOVA, $F = 16.281$, $df = 5, 308$, $P < 0.001$), inhibiting further comparison of complete datasets. Comparisons of subsets of the data, however, indicated that medusae of statistically similar sizes (38–45 mm, ANOVA,

Table 1 Swimming behavior of *Mastigias* medusae at noon during overcast-to-rainy weather. Medusae of similar sizes were studied in all locations (ANOVA $F_{3,80} = 0.542, P = 0.655$). *Asterisk* indicates significantly different mean angle of turn ($P < 0.05$) after sequential Bonferroni correction; 3 of 6 possible pairwise *t*-tests were selected to maximize power (Wilkinson et al. 1992:244)

	Location			
	OTM	RCA	BJLK	GLK
Mean (SE) medusa size (mm)	72.5 (5.34)	71.5 (4.35)	71.5 (4.64)	79.1 (5.62)
Percentage of turns[a] = 0	22.0	16.6	16.3	14.2
Mean (SE) angle of turn	24.9° (1.28°)	28.9° (1.56°)	36.8° (1.74°)	38.7° (2.04°)
n_1,n_2	20, 323	20, 355	23, 387	21, 324
Comparison...		vs OTM	vs RCA	vs BJLK
t-value		1.990	3.377	0.700
df		661	737	709
P (two-tailed)		0.047	< 0.001*	0.484

[a] "Turns" defined as difference in bearings at 10-s time intervals. A turn = 0 means no change in direction

n_1 Number of medusae observed. n_2 total number of turns measured

Medusae were not migrating strongly during these measurements because it was noon, the weather was heavily overcast, and medusae were well distributed across locations rather than strongly aggregated as on sunny days. Thus, swimming behavior is taken to reflect underlying differences in tendencies to turn

Table 2 Response of *Mastigias* medusae to noon-time shadows. The proportion of medusae that (1) avoided shadows, (2) entered shadows but subsequently turned and swam back into the light, or (3) showed no response during the period of observation is shown. Shadows were cast either by mangroves at the lakeside ([a]) or by a raft near the middle of the lake ([b])

	Location						
	RCA[a]	OLO[a]	GLK[b]	CLM[a]	OTM[b]	BJLK[a]	BJLK[b]
n	14	39	22	44	29	22	36
Avoided shade	0.57	0.36	0	0.45	0	0.36	0.11
Entered, turned, exited	0.43	0.54	0.95	0.55	0.90	0.64	0.89
No response	0	0.10	0.05	0	0.10	0	0

$F = 29.06$, $df = 5, 25$, $P = 0.519$; 115–135 mm, ANOVA, $F = 1.236$, $df = 5, 43$, $P = 0.309$) in different locations had statistically different pulse rates (38–45 mm, ANOVA, $F = 0.29.06$, $df = 5, 25$, $P < 0.001$; 115–135 mm, ANOVA, $F = 30.01$, $df = 5, 43$, $P < 0.001$; Table 3). Pulse rates also were influenced by ambient temperature ($r = 0.522$), being greater in CLM medusae (20–45 mm) at 30°C (2.72 ± 0.11, $n = 16$) than at 28°C (2.30 ± 0.11, $n = 16$) or 26°C (2.10 ± 0.11, $n = 16$; ANOVA, $F = 8.964$, $df = 2, 45$, $P = 0.001$).

7. Swimming speeds were variable (ANOVA, $F = 55.89$, $df = 5, 426$, $P < 0.001$). but, within sites, the distribution of swimming speeds was statistically invariant with bell diameter (Table 4). Swimming speeds segregated into three groups: [BJLK, GLK, OTM] < [CLM, OLO] < RCA (Tukey test, $df = 426$, $P < 0.001$). Across all sites, mean swimming speeds were positively correlated with mean pulse rates (38–45 mm class, $r = 0.769$, $n = 6$; 115–135 mm class, $r = 0.801$, $n = 6$).

Estimation of an historical rate of predation on Mastigias by anemones in OTM. In OTM, the anemone *Entacmaea medusivora* occurred at a mean density of 4.23 m^{-2} (SE 0.84 m^{-2}). However, individuals were patchily distributed, occurring in numbers as low as 0 m^{-2} to as high as 75 in a single quadrat (0.25 m^2). Assuming the perimeter of OTM is approximately 980 m, there were approximately 12,000 anemones in the top 3 m of the lake. Anemones were most dense on submerged prominences and fallen logs that extended beyond the shadowline cast by overhanging mangroves, where occasionally they catch *Mastigias*. In contrast, anemones generally were rarer in areas shaded by the mangroves. In BJLK, *Mastigias* bumped into areas of shallow benthos at the side of BJLK at an average rate of 5.9 medusae m^{-2} h^{-1} (SE 3.4 m^{-2} h^{-1}, $n = 3$, total 30 min observation) when the benthos was illuminated by the sun. In contrast, they were never observed bumping into the benthos when it was shaded (total 19 min observation, 25 m^2 area). The historical rate of predation by *E. medusivora* on *Mastigias* in OTM was estimated by integrating the distribution and abundance of anemones in OTM with the rate of encounters of *Mastigias* and benthos in BJLK. If a medusa of median size (bell diameter 2 cm, area 3.142 cm^2) impacted the benthos ~3,200 times, on average, it would hit 4.23 anemones. Thus, on average, a medusa would collide with an anemone once every ~130 h ($\approx 3{,}200/4.23/5.9$). Assuming *Mastigias* only collided with anemones in the top few meters during daylight, approximately 12,000 medusae would be consumed every 11 days, i.e. 400,000 medusae per year.

Discussion

Ancestral patterns and exaptation

Analogues of modern-day lagoonal environments likely existed in Palau and other western Pacific islands throughout the Late Pleistocene and Holocene (Pandolfi

Table 3 Pulse rates (pulses s^{-1}) of *Mastigias* related to bell diameter. Measurements were made at ambient water temperatures of approximately 29°C to 30°C. *d* Bell diameter in millimeters

	Location					
	RCA	OLO	GLK	CLM	OTM	BJLK
All size classes						
Rate =	$30.36\ d^{-0.604}$	$17.63\ d^{-0.506}$	$24.99\ d^{-0.606}$	$14.83\ d^{-0.493}$	$25.02\ d^{-0.649}$	$23.8\ d^{-0.615}$
r^2	0.959	0.878	0.959	0.962	0.979	0.957
n	18	50	53	59	78	62
38–45 mm class						
Mean ± SE	3.19 ± 0.07^{a}	2.54 ± 0.07^{bc}	2.57 ± 0.05^{b}	2.27 ± 0.07^{cd}	2.17 ± 0.07^{d}	2.54 ± 0.07^{bc}
n	5	8	8	5	5	4
115–135 mm class						
Mean ± SE	1.64 ± 0.04^{e}	1.46 ± 0.03^{f}	1.34 ± 0.03^{fg}	1.35 ± 0.03^{fg}	1.05 ± 0.03^{h}	1.25 ± 0.04^{g}
n	5	9	11	10	9	5

[a,b,c,d] Mean pulse rates of 38–45 mm medusae are significantly different (ANOVA: $F = 29.06$, $df = 5, 25$, $P < 0.001$), segregating into four, sometimes overlapping, groups ([a,b,c,d]) On the basis of post-hoc Tukey test ($df = 25, P < 0.05$)

[e,f,g,h] Mean pulse rates of 115–135 mm medusae are significantly different (ANOVA: $F = 30.01$, d.f. = 5, 43, $P < 0.001$), segregating into four, sometimes overlapping, groups ([e,f,g,h]) On the basis of post-hoc Tukey test ($df = 43$, $P < 0.05$)

Table 4 Swimming speeds (cm s^{-1}) of*Mastigias* related to bell diameter. Measurements were made at ambient water temperatures of approximately 29°C to 30°C. *d* Bell diameter in millimeters

	Location					
	RCA	OLO†	GLK	CLM^{e}	OTM^{f}	BJLK
Speed =	6.99–0.005 d	5.89–0.034 d	4.91–0.003 d	5.90–0.003 d	4.15 + 0.003 d	4.32 + 0.001 d
±95% CI	0.91, 0.010 d	0.57, 0.084 d	0.42, 0.005 d	0.93, 0.009 d	0.32, 0.003 d	0.43, 0.006 d
r^2	0.037	0.008	0.018	0.012	0.039	0.001
n	40	79	84	40	109	80
F	1.44	0.652	1.538	0.464	4.364	0.089
d.f.	1, 38	1, 77	1, 82	1, 38	1, 107	1, 78
P	0.24	0.42	0.22	0.50	0.04^{f}	0.77
Mean ± SE	6.5 ± 0.32^{a}	5.7 ± 0.11^{b}	4.7 ± 0.08^{c}	5.6 ± 0.16^{b}	4.5 ± 0.06^{c}	4.4 ± 0.08^{c}

^{a,b,c}Mean swimming speeds are significantly different (ANOVA: $F = 55.89$, $df = 5, 426$, $P < 0.001$) separated into three groups (^{a,b,c}) on the basis of post-hoc Tukey test ($df = 426$, $P < 0.001$)

^{e}Lines fitted to swimming speed data are approximations due to lack of precise data describing size of medusae. Assuming this does not influence significantly the fit of regressions, the slope of all regressions was statistically indistinguishable from zero after sequential Bonferroni correction across locations ($\alpha = 0.05$).

^{f}Linear regression is not significantly different from zero if the two largest (17 cm) medusae are excluded from analyses ($n = 107$, $F_{1,105} = 3.618$, $P = 0.06$) or if all medusae larger than 15 cm are excluded ($n = 101$, $F_{1,99} = 2.834$, $P = 0.1$) making the range of sizes more similar to those studied in other marine lakes

1996; Cabioch et al. 1999; Jackson and Johnson 2000). In contrast, Palau's marine lakes probably formed as the sea-level rose following the most recent glacial maximum (Hamner and Hamner 1998). Thus, whereas lagoonal populations probably have relatively ancient origins, historically large effective population sizes, and habitats that changed little over many decamillenia, marine lake populations likely are descendents of relatively few, recent, colonizers of novel peripatric marine environments. Thus, modern-day cove *Mastigias* likely retain characteristics reminiscent of *Mastigias* that occurred around Palau since the last glacial maximum while novel characters in marine lake populations are most likely derived (see, for analogy, Bell and Foster 1994; Foster et al. 1996). Therefore, six behavioral characteristics, ubiquitous among the populations of *Mastigias* studied, are symplesiomorphic:

1. the daily eastward component of all horizontal migrations,
2. shadow avoiding phototaxis, which is probably time-adjusted (Graham et al. 2001),
3. aggregation of *Mastigias* near the surface during the day and their redistribution throughout the water column during the night,
4. turning by medusae in the absence of other stimuli,
5. constant swimming speed across medusae of all sizes, despite
6. decreasing pulse rate with increasing bell diameter.

In some cases, symplesiomorphies may be under similar selection pressures in the marine lakes and in the coves. For example, eastward migrations start with aggregation in the west and finish with aggregation in the east, which exposes medusae and their photosymbiotic zooxanthellae to incident sunlight as early and as late as possible each day. Shadow avoidance keeps *Mastigias* and their zooxanthellae in direct sunlight for the interim. Thus, photosymbionts might be exposed optimally each day (Hamner and Hauri 1998). Another example is turning in the absence of other stimuli, which may have two benefits. First, it likely keeps *Mastigias* near a spot that already is proven good (e.g. in a patch of sunlight or food, in a breeding aggregation) or, at least, does not expose them to a situation that is considerably worse (e.g. in shade or sparse food, or alone; see Larson 1992; see also Purcell et al. 2000). Second, continuous turning in the horizontal plane coupled with principally vertical swimming at night will keep them in approximately the same place, i.e. in open water and away from the side, overnight (e.g. Fig. 2, OTM, also GLK 1600 cf. 2300 hours). The effects of turning, directed swimming, reflected light from the moon, and other factors on the westward redistribution of *Mastigias* each night (e.g. Fig. 2, RCA, GLK, BJLK) is yet to be investigated.

In other cases, symplesiomorphies may be canalized trade-offs. For example, due to hydrodynamic considerations, growth of medusae inevitably leads to decreased pulse rates in the absence of large increases in muscle mass. This trade-off may be of little consequence because swimming speed does not decrease and feeding potential (i.e. clearance rate) necessarily still increases.

Other symplesiomorphies may be exaptations. For example, vertical migration is an ancestral characteristic exhibited by *Mastigias* in holomictic coves and lakes that may reduce advection of individuals away from the breeding population by surface currents when directional light cues are weak or absent, for example at night or during storms (this study; see also Uchida 1926; Siebeck 1980; Eiane et al. 1998). In novel meromictic marine lakes, vertical migration also is beneficial in allowing *Mastigias* to exploit a novel source of nutrients at the chemocline (Hamner et al. 1982; Muscatine and Marian 1982).

However, it is possible that vertical migration in meromictic lakes was originally (and still may be in some circumstances) deleterious, because it exposes medusae to dysoxia, anoxia, and toxic hydrogen sulfide (see Dawson 2000). Whether *Mastigias* evolved novel or modified existing sensory capabilities or metabolic processes to deal with this potential hazard is not known, but aquarium observations indicate that responses to salinity, temperature, density, light, and hydrogen sulfide, or some combination thereof, may be involved (Dawson 2000).

Geographic variation and behavioral evolution in *Mastigias*

Although most of the behavioral characters discussed herein are symplesiomorphic, all character-states vary geographically. Derived character-states include slower pulse rates, slower swimming speeds, altered frequency and angle of turns made in the absence of other stimuli, deeper distributions during the day, diffuse horizontal distributions, and bimodal horizontal distribution in lake populations, as explicated below. The evolution of behaviors that lead to diffuse or bimodal horizontal distributions may be closely related to the evolution of one quite novel character—westward migration during late-morning and afternoon—which occurs in at least two marine lakes. Given the geography of Palau, such changes likely occurred independently and in parallel in each lake, presenting the strongest possible natural evidence of evolution by natural selection and adaptation (Foster 1999).

1. Pulse rate. The rate of decrease in pulse rate with increasing medusa size is unique to each population of *Mastigias*. Pulse rates per se also differ significantly among populations. Most notably, pulse rates of cove *Mastigias* are significantly faster than pulse rates of lake *Mastigias*, a difference that withstands even overly conservative correction for temperature differences (± 0.4 pulses s^{-1} for a 2°C change in 20–45 mm medusae). Pulse rates also differ among marine lake populations, but the relationship of pulse rate to bell diameter produces variable and inconsistent differences between populations when considered across all sizes of medusae. This suggests that, at least among lakes, pulse rates are not strongly selected. If there is an advantage in pulse rate differences between coves and lakes, however, it likely is a function of its influence on swimming speed ($r \approx 0.8$).

2. Swimming speed. Swimming speed is significantly faster in RCA than in all marine lakes, and significantly faster in OLO and CLM than in BJLK, GLK, or OTM. Thus, swimming speed appears to be under relaxed selection pressure (or selection for slower speeds) in marine lakes. The difference between RCA and the marine lakes may be related to the chance of advection away from the breeding population (e.g. Hamner et al. 1994; Eiane et al. 1998), which is much greater in RCA than the lakes because of its greater tidal exchange (Hamner and Hamner 1998), more open habitat, and modest surface connection to oceanic waters. Many medusae have been seen just outside RCA, but no marine lake medusae have ever been seen outside the lakes (pers. obs.). The differences in swimming speeds of jellyfish among marine lakes also may be related to the duration of (reduced) selection on medusae, i.e. time since isolation. For example, BJLK is 35 m deep and hypothetically formed about 5,000 years earlier than OLO, which is only 4 m deep (see Dawson 1992:224; Kayanne et al. 2002); *Mastigias* in OTM, which is 30 m deep, also swim slower than those in OLO, although they migrate farther. If this is the case, the occurrence of uncommonly fast (for marine lake medusae) *Mastigias* in CLM may be explained by sporadic strobilation in CLM (Dawson et al. 2001) which leads to inconsistent, and often zero, selection pressure on medusae from year to year (Dawson et al. 2001).

3. Turning behavior. In contrast to pulse rates and swimming speed, there is no clear difference in turning behavior between cove and marine lake *Mastigias*, nor an apparent relationship between turning behavior and time since isolation (assuming time since isolation is proportional to lake depth). Medusae in OTM turned least, suggesting an inverse relationship between the tendency to turn and the distance and cohesiveness of migration. The size and frequency of turns may also be related to the openness of the habitat or distribution of resources (e.g. food, light) and strategies for their exploitation.

4. Vertical distribution. Daytime aggregation of *Mastigias* at depth is specific to meromictic lake populations, which suggests meromictic lake medusae are more sensitive than cove and OLO medusae to light intensity. This is consistent with meromictic marine lake *Mastigias* migrating shallower when sunlight is ameliorated (see also Siebeck 1980). However, differences in noon WMD between lakes are not related to differences in the sensitivity of jellyfish, but rather to differences in water clarity and light penetration. The noon WMD of marine lake *Mastigias* is highly positively correlated with Secchi disc depth ($r = 0.9585$). Thus differences in WMD between lakes and times may reflect behavioral strategies to mitigate photoinhibition or UV damage that replace the protection that pigmentation may afford cove *Mastigias* (e.g. Hairston 1980; Rhode et al. 2001; see "Behavior and morphology"). Vertical migration by *Mastigias* does not track prey distributions (Hamner et al. 1982).

5. Diffuse horizontal distribution. Theoretically, diffuse horizontal distributions could result from weak horizontal migrations contributed to by greater turning, slower swimming, various effects of basin topography, or the overlap of two different migrational patterns (west-to-east and east-to-west). However, none of these is sufficient to explain diffuse distributions such as that observed in BJLK. *Mastigias* in BJLK do not turn the most and could swim across the lake in several hours, basin topography should permit reasonably cohesive migrations (because it permits early-morning and late-afternoon sunlight with large horizontal components to strike the lake), and overlap of two migrational patterns could equally generate a strongly bimodal distribution.

Thus, it seems likely that diffuse migration, as epitomized in BJLK, is itself another character state due, in part, to a lack of strong selection for behaviors that generate more cohesive migrations in the coves and other lakes. The lack of strong selection may result from reduced predation, weaker currents, more plentiful sunlight (e.g. BJLK 76% cf. BJCK 61% of daily total), and abundant food (e.g. Hamner et al. 1982).

6. *Bimodal horizontal distribution*. Bimodal distributions in BJLK (noon) and CLM (0900 hours) resulted largely from opposing west-east biases in the densities of small and large medusae [different size medusae also segregate along a north-south axis in GLK (personal observation.); see also Hamner et al. 1982]. Spatial sorting of size classes may reduce mechanistic interference (Hamner et al. 1982) and shading of small medusae by large medusae (small medusae have frequently been observed trapped inside the bell of large medusae in OTM; pers. obs.). In CLM, the rapid, early morning, eastward migration of small medusae, followed by westward migration also may reduce mortality attributable to predation by *Entacmaea medusivora* on pre-reproductive medusae (medusae mature at 6–7 cm, unpubl. data).

7. *Afternoon westward migration*. Perhaps of greatest evolutionary interest is the alteration of the timing and direction of migration by *Mastigias* in OTM (see also CLM), which may be one of the few times a "derived, complex behavioral pattern" has been detected in population comparisons (see Foster 1999). Although all populations migrate eastward in the morning, the novel, highly cohesive, westward afternoon migration in OTM is quite probably attributable to predation by the sympatric anemone, *E. medusivora*, of which over 12,000 occurred in the top few meters of OTM where *Mastigias* swim during the day (Hamner et al. 1982; this study). Anemones also occur in CLM, where *Mastigias* migrate similarly to those in OTM, but not in the other lakes discussed here. Presently, hundreds of medusae per year are eaten by *E. medusivora*, usually on sunlit promontories and fallen trees, yet historically predation probably was much greater causing hundreds of thousands of mortalities per year in a population of only a few million medusae. These exceptionally high predation levels can have only one of two results: extirpation of the population or evolution of novel behavior that reduces mortality. By migrating east in the morning and west in the afternoon (i.e. toward the sun) *Mastigias* in OTM always aggregate against or near to a shadowline cast by the surrounding hills and vegetation rather than against the illuminated benthos, as they do in BJCK and GLK for example, and thus OTM *Mastigias* minimize encounters with *E. medusivora*. Positive phototactic behavior also has been demonstrated in the freshwater zooplankter *Chaoborus punctipennis* in response to the presence of predatory fish in an effectively benthic situation (i.e. in compartments at the ends of experimental chambers; O'Bryan and Forrester 1997). The anti-predatory behavior of *Mastigias* also may involve "avoidance of shore" (sensu Siebeck 1980; see Hamner and Hauri 1981), although medusae do not aggregate near the middle of coves or lakes, as would be expected of pelagic animals given the high surrounding topography (e.g. Fig. 3 of Siebeck 1980), nor respond in a manner typical of littoral copepods (e.g. Fig. 7 of Siebeck 1980) as *Mastigias* appear to avoid shadows rather than the "dark field" caused by local topography and may even aggregate against an illuminated benthos. These idiosyncrasies suggest a suite of interacting influences and mechanisms which strike different balances in different populations (Hamner and Hauri 1981). In this context, historically high predation by anemones seems to be the difference that is most likely to explain why *Mastigias* in OTM (and CLM), in contrast to those in coves, GLK, and BJLK, exhibit a migration pattern that is sub-optimal when considered only in terms of the duration of irradiance of zooxanthellae because afternoon westward migration causes medusae to swim into the advancing shadows rather than waiting to catch the last rays of the sun (Hamner and Hauri 1981).

Behavior and morphology

In some cases, behavioral evolution in *Mastigias* seems likely to be closely related to morphological evolution, as has been found in other species (e.g. Podos 2001). For example, differences in pulse rates and swimming speeds may correspond to differences in the structure of the bell, which is the major organ of propulsion, or to other morphological differences such as the shapes of the oral arms and terminal clubs (Dawson, submitted) that may alter drag. Differences in sensitivity to light also may be related to morphological changes. Cove and OLO *Mastigias*, which bask in surface waters on the brightest of days, often are heavily pigmented with opaque spots and a translucent blue that is similar to cassio blue which occurs in another rhizostome, *Cassiopea*, and may be photoprotective (Arai 1997; but see Blanquet and Phelan 1987). In contrast, the barely pigmented *Mastigias* in the meromictic lakes migrate deeper into murky water as sunlight gets brighter, apparently trading morphological for behavioral protection (see also Hairston 1980). The relative benefits of morphological and behavioral photoprotection in *Mastigias* are unknown, but the latter potentially allows finer and more immediate control of exposure and may, therefore, have been under positive selection. However, the cause of reduced pigmentation in marine lake *Mastigias* is not known, and it may also be related to changes in food, other resources, or metabolism that preceded, co-occurred with, or followed, the modification of vertical migratory behavior.

Geographic variation, behavior, and evolution in marine plankton

Geographic variation in the behavior of *Mastigias* in Palau is a result of rapid evolution effected by geographic isolation and novel selective environments. The behavioral differences have parallels in morphological differences (Dawson, submitted) which, in turn, have parallels in *Mastigias* isolated in marine lakes elsewhere (Tomascik et al. 1997). Population differentiation also is evident in other scyphomedusae, including *Aurelia* (Dawson and Martin 2001), *Cyanea* (Brewer 1991), and *Catostylus mosaicus* (Pitt and Kingsford 2000). The implication is that geographic variation and local adaptation may be common among neritic jellyfishes and probably more common than traditionally thought in other coastal plankton (see Dawson and Martin 2001) and other marine invertebrates (e.g. Scapini et al. 1999; Saigusa 2001). The emerging picture is one of heterogeneity and complexity more akin to images of terrestrial and freshwater environments than traditional perceptions of the marine environment. Concomitantly, phylogeographic structure in coastal species is being reported increasingly frequently (e.g. Avise 1992; Benzie 1998).

The origin of phylogeographic structure in coastal marine taxa often is linked to glacial periods of reduced sea-level that temporarily increase geographic isolation (e.g. Avise 1992; Benzie 1998). This suggests that divergence is rapid, on the order of several to tens of thousands of years considering patterns of Pleistocene sea-level change (e.g. Dawson 1992), and sufficiently large to inhibit genetic homogenization during subsequent periods of higher sea-level and greater gene flow. Commensurately, studies of fossil assemblages in continental shelf deposits indicate that punctuational patterns of speciation predominate in marine invertebrates (Benton and Pearson 2001). The suggestion that behavioral differences are good indicators of the early stages of speciation and of sub-species (Wcislo 1989; Foster et al. 1996; Foster 1999) therefore suggests that the rapid evolution of behavior in marine plankton may have an important role in the generation of coastal biodiversity. As such, additional comparative behavioral studies on populations of coastal marine plankton should be of interest to ethologists, evolutionary biologists, biogeographers, and marine conservation biologists.

Concluding remarks

Despite increasing evidence of behavioral complexity and geographic isolation, and technological advances that have enabled in situ studies, ethological studies of marine zooplankton are still relatively rare (Graham et al. 2001). In particular, comparative studies are lacking because of difficulties in identifying distinct populations that can be visited repeatedly (Hamner 1985). In this light, marine lakes and coves in Palau and analogous systems elsewhere are extremely valuable because they provide the "ideal scenario, in which parallel, independent evolution of [behaviors] can be inferred from...geographical data" and thus offer "promising opportunities for testing...optimality models" and hypotheses regarding evolution in the marine environment (Foster 1999:190–191; see also Hamner and Hauri 1981). However, uncontrollable variation between sites and times can complicate studies in natural environments and additional information may be required to demonstrate selection and establish causal relationships (Foster 1999). Thus, comparative field studies are best integrated with theory, modeling, and experimentation (Graham et al. 2001). Advances in our understanding of the evolution of behavior and geographic variation in marine plankton also will be facilitated by developing appropriate laboratory-based techniques (Hamner et al. 1995; Dawson 2000) including simulations and experiments that mimic and manipulate naturally occurring environmental variation (e.g. O'Bryan and Forrester 1997; Strand et al. 2002).

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