# Density- and trait-mediated top–down effects modify bottom–up control of a highly endemic tropical aquatic food web

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Benthic invertebrates mediate bottom–up and top–down influences in aquatic food webs, and changes in the abundance or traits of invertebrates can alter the strength of top–down effects. Studies assessing the role of invertebrate abundance and behavior as controls on food web structure are rare at the whole ecosystem scale. Here we use a comparative approach to investigate bottom–up and top–down influences on whole anchialine pond ecosystems in coastal Hawai'i. In these ponds, a single species of endemic atyid shrimp (*Halocaridina rubra*) is believed to structure epilithon communities. Many Hawaiian anchialine ponds and their endemic fauna, however, have been greatly altered by bottom–up (increased nutrient enrichment) and top–down (introduced fish predators) disturbances from human development. We present the results of a survey of dissolved nutrient concentrations, epilithon biomass and composition, and *H. rubra* abundance and behavior in anchialine ponds with and without invasive predatory fish along a nutrient concentration gradient on the North Kona coast of Hawai'i. We use linear models to assess 1) the effects of nutrient loading and fish introductions on pond food web structure and 2) the role of shrimp density and behavior in effecting that change. We find evidence for bottom–up food web control, in that nutrients were associated with increased epilithon biomass, autotrophy and nutrient content as well as increased abundance and size of *H. rubra*. We also find evidence for top–down control, as ponds with invasive predatory fish had higher epilithon biomass, productivity, and nutrient content. Top–down effects were transmitted by both altered *H. rubra* abundance, which changed the biomass of epilithon, and *H. rubra* behavior, which changed the composition of the epilithon. Our study extends experimental findings on bottom–up and top–down control to the whole ecosystem scale and finds evidence for qualitatively different effects of trait- and density-mediated change in top–down influences.

Benthic invertebrates are important mediators of top–down and bottom–up influences in many aquatic food webs (Wallace and Webster 1996). Increased productivity at the base of the food web can increase benthic invertebrate abundance (Gruner 2004, Slavik et al. 2004, Cross et al. 2006), whereas predators can reduce invertebrate abundance through consumption (Obernborfer et al. 1984, Gilliam et al. 1989). Herbivorous invertebrates also act as agents of top–down control, as their feeding alters the abundance, diversity and productivity of photosynthetic organisms (Rosemond and Mulholland 1993, Hillebrand and Kahlert 2001). The strength of this top–down effect is itself sensitive to both top–down and bottom–up change in food webs: increased resource abundance decreases the foraging rate of invertebrates (Anholt and Werner 1995, 1998), and predation risk reduces the foraging rate and time spent in vulnerable habitat by consumers

(Zaret and Suffern 1976, Mittelbach 1981, Parkos and Wahl 2010). Such trait-driven change in top–down control may be especially important in isolated ecosystems, where behavioral or biomass compensation by other members of the community may be constrained by a lack of diversity (Loreau et al. 2002, Duffy 2002, Covich et al. 2004).

Despite the potential importance of trait-mediated top–down control, its strength has rarely been assessed at the whole ecosystem scale, especially relative to density-mediated or bottom–up influences. Most of the few whole-ecosystem studies contrasting top–down and bottom–up control do not attempt to partition trait- from density-mediated effects (Carpenter et al. 1996, Jeppesen et al. 2000, 2003, Norlin et al. 2005, Hambright et al. 2007, Hoekman 2011, Friederichs et al. 2011). Nearly all studies contrasting trait and density-mediated effects of consumers,

moreover, rely on experimental mesocosms or microcosms that are subject to design-specific effects, and many do not seek to contrast top–down and bottom–up influences (Trussell et al. 2006, Werner and Peacor 2006, reviewed by Werner and Peacor 2003, Schmitz et al. 2004). Expanding evidence to whole ecosystem studies that assess both trait and density-mediated top–down control relative to bottom–up influences is important for understanding how ecosystems may respond to changes in abiotic and biotic conditions.

Hawaiian anchialine ponds are land-locked, mixohaline pools that are tidally influenced by subsurface watertable links (Holthuis 1973, Brock et al. 1987) and are ideal systems for assessing the long term influence of top–down and bottom–up changes to low diversity food webs. The invertebrate biomass of these ponds is dominated by a single endemic atyid shrimp *Halocaridina rubra*, the grazing of which is thought to maintain the short-turfed, diverse epilithon typical of intact anchialine ponds (Bailey-Brock and Brock 1993). Deep accumulations of organic matter, however, have replaced this epilithon 'lawn' in ponds near human development (Bailey-Brock and Brock 1993, Wiegner et al. 2006). This change may stem from a bottom–up effect, nutrient loading that has occurred since the construction of nearby resorts in the mid 1990s (Wiegner et al. 2006), or a top–down effect, the introduction of predatory non-native poeciliid fish. These top predators may reduce grazing pressure by reducing the numerical abundance of *H. rubra* through consumption or by reducing the time *H. rubra* spends feeding, releasing the epilithon from top–down control (Capps et al. 2009, Carey et al. 2010, MacKenzie and Bruland 2011). These ponds thus provide a 'natural' experiment for the study of whole-ecosystem responses to the long-term modification of bottom–up and top–down control in highly endemic food webs and provide an opportunity for insight into the relative influence of trait and density-mediated top–down effects.

Here we present the results of a comparative field survey of epilithon biomass and nutrient content, as well as *H. rubra* abundance and behavior in 10 anchialine ponds with and 10 without invasive poeciliid fish across an established nutrient concentration gradient along the North Kona coast of Hawai'i island. The goal of this survey is to assess, at the whole-ecosystem scale, the relative strengths of these forces in structuring food web dynamics and to enable managers to account for these forces in making decisions that buffer anchialine ponds from human development.

## Methods

### Site description

In January 2011, we surveyed 20 anchialine pools selected from among several hundred known ponds along the North Kona coast of Hawai'i Island (Fig. 1, Table 1). All ponds were within 100–250 m of the shoreline and were selected based on the presence or absence of human development along each pond's immediate periphery and the presence or absence of invasive poeciliid fish. Seven of the surveyed ponds had human development on their immediate periphery and thirteen had no development within several hundred meters (often much more). The seven ponds near human development were located on
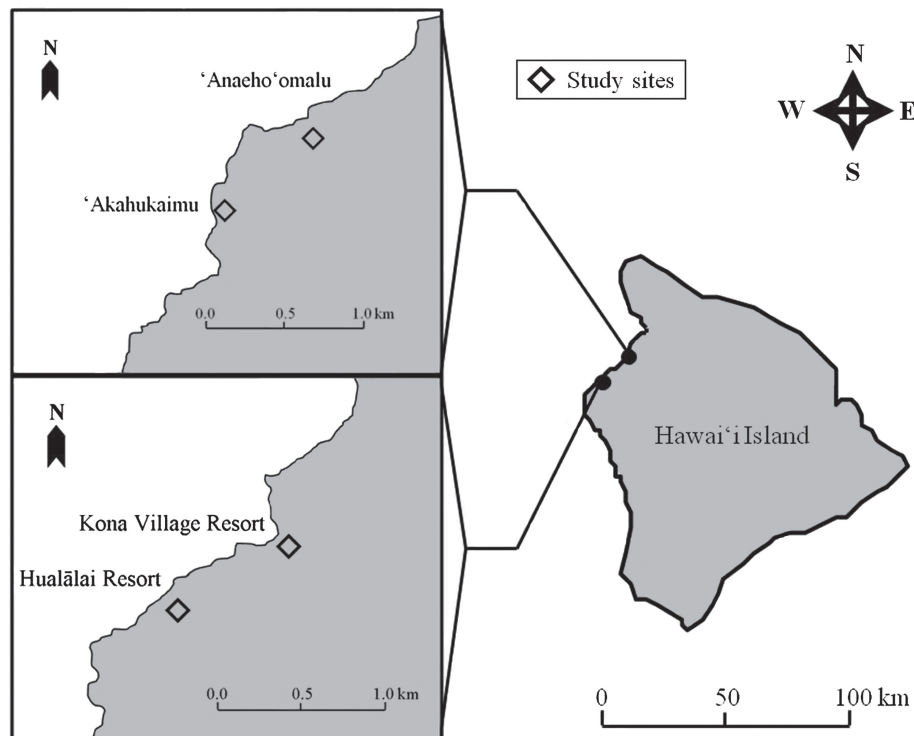


Figure 1. Map of the North Kona Coast on the island of Hawai'i showing the four study site locations.

Table 1. List of ponds surveyed and their environmental properties.

| Site | Pool name | Habitat periphery | Surface area (m²) | Max depth (m) | Mean NO$_2^-$ + NO$_3^-$ (μM) | Mean NH$_4^+$ (μM) | Mean DIN (μM) | Mean SRP (μM) |
|------|-----------|-------------------|-------------------|---------------|-------------------------------|--------------------|---------------|---------------|
| Fish | | | | | | | | |
| ʻAnaehoʻomalu | AB12 | undeveloped | 36.2 | 0.5 | 50.99 | 1.90 | 52.90 | 1.88 |
| ʻAnaehoʻomalu | AB14 | undeveloped | 62.8 | 0.5 | 58.51 | 1.78 | 60.29 | 2.11 |
| ʻAnaehoʻomalu | AB16 | undeveloped | 3.4 | —* | 50.38 | 2.73 | 53.11 | 1.90 |
| ʻAnaehoʻomalu | AB17 | undeveloped | 2.5 | — | 51.03 | 2.00 | 53.03 | 1.90 |
| ʻAnaehoʻomalu | AB18 | undeveloped | 8.3 | — | 55.99 | 1.93 | 57.92 | 2.01 |
| ʻAnaehoʻomalu | AB5 | undeveloped | 2.9 | 0.6 | 58.27 | 3.40 | 61.68 | 2.15 |
| ʻAnaehoʻomalu | AB6 | undeveloped | 104.9 | 0.8 | 54.65 | 1.97 | 56.62 | 1.97 |
| Hualālai | Waiiki | developed | 77.2 | — | 145.61 | 3.09 | 148.70 | 5.53 |
| Kona Village | KVBP | developed | 30.9 | 0.6 | 109.84 | 9.06 | 118.89 | 7.93 |
| Kona Village | KVPM4 | developed | 128.7 | 0.3 | 105.66 | 3.82 | 109.48 | 9.27 |
| No fish | | | | | | | | |
| ʻAnaehoʻomalu | AB11 | undeveloped | 1.8 | 0.2 | 56.73 | 2.28 | 59.01 | 2.01 |
| ʻAnaehoʻomalu | AB13 | undeveloped | 9.5 | — | 58.27 | 1.97 | 60.24 | 2.13 |
| ʻAnaehoʻomalu | AB15 | undeveloped | 6.7 | 0.35 | 58.60 | 2.01 | 60.61 | 2.20 |
| Hualālai | Hoʻonanea | developed | 431.2 | — | 125.46 | 3.15 | 128.61 | 7.99 |
| Hualālai | Waiʻolu | developed | 282.7 | — | 162.43 | 3.98 | 166.41 | 6.91 |
| Hualālai | Wahi Pana | developed | 1.1 | — | 181.75 | 2.25 | 183.99 | 6.00 |
| Kona Village | KVWB | developed | 1.2 | 0.5 | 77.51 | 6.09 | 83.60 | 7.53 |
| ʻAkahukaimu | WW10 | undeveloped | 8.7 | — | 76.59 | 1.52 | 78.10 | 1.72 |
| ʻAkahukaimu | WW2 | undeveloped | 32.8 | 0.6 | 80.10 | 1.51 | 8.61 | 1.59 |
| ʻAkahukaimu | WW5 | undeveloped | 202.8 | 1.25 | 74.52 | 1.62 | 76.14 | 1.79 |

*line indicates pond was shallower than 0.2 m.

resort properties (Hualālai Four Seasons and Kona Village) that are managed to maintain native species diversity but nevertheless had associated high density housing, restaurants and golf courses in their immediate vicinity. Three contained invasive fish. Ponds without human development on their immediate periphery were located in two relatively undeveloped watersheds (Puʻuanahulu and ʻAnaehoʻomalu). Seven of these 13 ponds contained invasive poeciliids. These ponds were surrounded by the plants: milo *Thespisia populnea*, ʻakulikuli *Sesuvium portulacastrum*, kiawe *Prosopis pallida*, and fountain grass *Pennisetum setaceum*. Pond AB6 exhibited a substantial covering of native ditchgrass *Ruppia maritima* and a cyanobacteria surface scum. At high tide, pond depths were generally less than 1 m (Table 1) and salinities were less than 7 g l$^{-1}$, indicating that our study ponds receive substantial groundwater inputs. The one exception was pond KVPM4 at Kona Village, which had a higher salinity level (14 g l$^{-1}$) due to deep well-water pumping to control the invasive cane toad *Bufo marinus* (D. Chai pers. comm.). Benthic substrates were comprised of basalt.

## Nutrient concentration

Concentrations of ammonium ($NH_4^+$), dissolved inorganic nitrogen (DIN; $NH_4^+ + NO_3^- + NO_2^-$) and soluble reactive phosphorus (SRP) were measured in each pond at both high and low tide. Salinity measurements were made concurrent with nutrient samples to compare ground and ocean water influence on the systems. Salinity measurements were made using a YSI model 85 with data logger. Water samples were drawn directly above the benthos, vacuum filtered through a 0.7 μm (GF/F Whatman) filter, placed on ice, and frozen within 6 h of sampling. Concentrations of nutrients were measured on an autoanalyzer

using standard methods: $NO_3^- + NO_2^-$ (detection limit (DL): 0.1 μmol l$^{-1}$, USEPA 353.4), $NH_4^+$ (DL: l μmol l$^{-1}$ USGS I-2525), and SRP (DL: 0.1 μmol l$^{-1}$, USEPA 365.5). Nutrient analyses were conducted at the Analytical Laboratory at the Univ. of Hawaiʻi at Hilo. The means of single high and low tide nutrient measurements at each pond were used for statistical analyses. Other studies of nutrients in Hawaiian anchialine ponds have indicated that DIN and SRP constitute the majority of total nitrogen (TN; mean = 85%) and total phosphorus (TP; mean = 87%) and are strongly correlated with TN and TP ($r^2 > 0.95$; B. Dudley, unpubl., Wiegner et al. 2006). Here we report values for inorganic nutrient concentrations only.

## Algal standing stocks

We measured area-specific chlorophyll *a* mass (chl *a*), ash-free dry mass (AFDM), and carbon:nitrogen (C:N) stoichiometry to assess standing stocks of epilithon in each pond. We used a Loeb sampler (Steinman and Lamberti 1996) to scrape clean an area of 4.9 cm$^2$ of benthic substrate at four locations along each of two transects per pond. The four scrapings from each transect were pooled and homogenized. A subsample from each pooled sample was then filtered for chl *a* on 25 mm GF/F filters, AFDM on pre-weighed and ashed 47 mm GF/F filters, and C:N on pre-weighed and ashed 25 mm GF/F filters.

Chl *a* concentration was used as a proxy for biomass of photosynthetic cells (Wayman 1975). We extracted chl *a* filters in 10 ml of 90% buffered ethanol at 4°C overnight in the dark. After 24 h, the extract was analyzed using a fluorometer (Hambright et al. 2007). To correct for phaeophytin, 10 μl of 0.1 N HCl was added to the extract after the initial reading and incubated at room temperature in the dark for two minutes before a second, acidified

reading was made. We calculated the concentration of chl *a* in the extract using a standard calibration of chl *a* versus fluorescence after the acidification correction for phaeophytin. AFDM filters were dried for 48 h at 55°C and weighed on an analytical balance. Each filter was then ashed at 450°C for 4 h and reweighed. AFDM was calculated as the difference between the weight of the dried sample with filter and the ashed filter with sample (Steinman and Lamberti 1996). Chl *a* and AFDM per area were then calculated by correcting for the volume sub-sampled from the beaker onto the filter, the total volume of the four pooled Loeb samples from the transect and the total area scraped by the four Loeb samples.

We used the autotrophic index (AI), a ratio representing the proportion of epilithic organic matter composed of actively photosynthesizing algal cells and calculated as (chl *a* per area)/(AFDM per area), to assess the trophic nature of the epilithon (Stevenson and Bahls 1999). High AI values indicate a more autotrophic epilithon, whereas low AI values indicate increased heterotrophy, assuming that increased standing stocks of non-photosynthesizing organic matter serve as a proxy for biomass of heterotrophic cells (Weitzel 1979). Filters to be analyzed for C:N were dried for 48 h at 55°C, rolled in tin and combusted in an elemental analyzer.

### H. rubra and fish presence

Because *H. rubra* makes pronounced diel migrations between epigeal (surface exposed) and hypogeal (subterranean) habitats (Capps et al. 2009, Carey et al. 2010), we estimated the abundance of *H. rubra* in each pond during both day and night using the catch per unit effort (CPUE) method of Capps et al. (2009). CPUE measurements were made at four locations along each of two transects in each pond, using a 20 cm wide, 0.5 cm mesh net for large ponds ($> 31$ m$^2$), and a 10 cm wide, 0.5 cm mesh net for all other, smaller ponds. Sweep duration was 20 s, except at KVWB (night) and Ho'onanea (day) where extremely high *H. rubra* densities were encountered and sweeps were shortened to five seconds. Counts were corrected for the size of the net and time of the sweep by either dividing the total count by the effort (sweep time × width of net; Capps et al. 2009) to create a CPUE index or by using effort as the weights argument of a generalized linear model (Crawley 2005). We measured carapace lengths from the tip of the rostrum to the posterior edge of the cephalothorax of ~20 *H. rubra* collected from each pond within 12 h of collection. *H. rubra* were then dried at 55°C for 48 h and weighed to the nearest mg.

We used minnow traps with fish bait to determine the identity of the poeciliids in each pond. We placed a minnow trap at the middle of each fish pond for 0.67 h. All fish were identified as guppies *Poecilia reticulata*, except those in Waiiki, which were identified as *Poecilia* spp. (possible hybrid between *P. mexicana* and *P. salvatoris*; Yamamoto and Tagawa 2000) and those in AB12, which were a combination of *P. reticulata* and *Gambusia affinis* (J. P. Friel pers. comm.). Specimens have been deposited at the Cornell Univ. Museum of Vertebrates.

### Data analysis

High and low-tide measurements of each nutrient were averaged, log-transformed to conform with the assumption of normality, and analyzed using linear models with fish presence and immediate human development on shoreline as factors. Chl *a*, AFDM, AI and C:N of epilithon samples were averaged across transects in each pond. Average *H. rubra* length was calculated for each pond based on all measured *H. rubra* from that pond.

We analyzed top–down and bottom–up influences on the epilithon using linear regressions with nutrient concentration and the presence of fish as predictors for pond-level averages of chl *a*, AFDM, AI and C:N. Nutrient concentration data were log-transformed to conform to the assumptions of normality. We then analyzed density-mediated and trait-mediated effects of top–down control on the epilithon by *H. rubra* by replacing fish as a predictor in linear models with two separate factors: the abundance of *H. rubra* (CPUE) and the extent of diel migration assessed as the difference between night and day CPUE divided by the night CPUE. The latter measure estimates what fraction of *H. rubra* density present at night migrated from outside the sampling area (presumably hypogeal habitat). The abundance of *H. rubra* in each pond was modeled as the nighttime CPUE.

Fish and nutrient effects on *H. rubra* were analyzed using linear models with nutrient concentrations and fish presence as predictors in each model. Individual counts of *H. rubra* were analyzed using a generalized linear mixed model with a Poisson distribution, using pond as a random factor and the weights function to correct for the number, time and net size of samples (Crawley 2005). Total CPUE estimates for each pond at day and night were square-root-transformed and analyzed using a linear model. Diel migration by *H. rubra* was analyzed using a generalized linear model with a binomial distribution and total day and night CPUE to weight the model (Crawley 2005). The assumptions of all linear models were confirmed using the gvlma function of R statistical software (Pena and Slate 2006). We used corrected Akaike information criterion (AICc) scores to determine which factors best explained variance in each measured variable (Akaike 1974).

The separate and combined magnitude of nutrient and fish effects were assessed using Cohen's *d*, a variance-standardized mean difference measure (Cohen 1988, Nakagawa and Cuthill 2007). Because Cohen's *d* can only be calculated based on categorical variables, we categorized each pond as either 'high' or 'low' in dissolved nutrient concentration. To make this categorization, we calculated a composite nutrient index by transforming DIN and SRP measurements to scale from zero (minimum value for that nutrient) to one (maximum value for that nutrient). We did not separately include $NH_4^+$ in this analysis because $NH_4^+$ is included in DIN (however, performing the analysis with $NH_4^+$ alone yielded the same categorization of high and low nutrient status ponds). Summing index scores for DIN and SRP created an index (ranging from zero to two) of overall nutrient abundance weighing nitrogen and phosphorus equally (because we make no a priori assumptions about which

nutrient may be limiting in the system). This composite index was bimodally distributed, with 'high nutrient' ponds having scores > 1.0 and 'low nutrient' ponds having scores < 0.2. Our twenty ponds could then be categorized into one of four 'treatments': 1) low nutrient, no-fish, 2) high nutrient, no-fish, 3) low nutrient, with fish and 4) high nutrient, with fish.

The effect size of nutrients alone was assessed by comparing the means of high and low nutrient ponds that did not have fish (i.e. contrasting treatments 2 and 1). Fish effect size was assessed by comparing means of ponds with fish to ponds without fish for low nutrient ponds only (i.e. contrasting treatments 3 and 1). The combined effect of fish and nutrients was assessed by comparing means of high nutrient fish ponds to those of low nutrient no-fish ponds (i.e. contrasting treatments 4 and 1). We divided differences in means among treatments in the specified contrast by the pooled standard deviation for that contrast to calculate the effect size (Nakagawa and Cuthill 2007) with those greater than 1 considered 'large' effects. All statistics were performed using R statistical software. We interpreted p values < 0.05 as significant and 0.05–0.10 as marginally significant.

## Results

### Land use and nutrient concentration

Ponds with human development on their immediate periphery had significantly higher concentrations of $NH_4^+$, DIN and SRP than ponds without human development (development effect on log-transformed nutrients in two-way ANOVA with fish and development as factors: $NH_4^+$: F = 23.46, p = 0.0001; DIN: F = 68.51, p < $10^{-6}$; SRP: F = 436.77, p < $10^{-13}$). Ponds with fish had higher DIN concentrations than those without fish (F = 7.445, p = 0.02), but neither $NH_4^+$ (F = 1.07, p = 0.32) nor SRP (F = 2.00, p = 0.18) differed between fish and no-fish ponds. The tide-level at the time of sampling did not significantly affect the concentration of any measured nutrient. Both $NH_4^+$ and DIN were positively correlated with SRP ($NH_4^+$: $r^2$ = 0.63, slope = 0.57, p < 0.0001; DIN: $r^2$ = 0.66, slope = 0.51, p < 0.0001), and $NH_4^+$ was positively related to DIN ($r^2$ = 0.21, slope = 0.53, p = 0.04).

### Bottom–up effects

ANOVA for linear models of epilithon measurements using SRP or $NH_4^+$ yielded very similar results. Here we mainly present the results of $NH_4^+$. The equivalent analyses with SRP and DIN are summarized in supplemental electronic materials (Supplementary material Appendix A1 Table A1, Fig. A1–A2) as are analogous analyses based on information theoretic model comparison (Supplementary material Appendix A1 Table A2). Results of model selection generally were consistent with results of ANOVA. A single, high $NH_4^+$ pond was tested for leverage in linear models, but this point was found to not exert strong influence on the results of either model selection or ANOVA.

Table 2. ANOVA of linear models for epilithon and two *H. rubra* measurements (significant results bolded, marginally significant results in italics). Nutrient is $ln(NH_4^+)$, see Supplementary material Appendix A1 Table A1 for DIN and SRP. All three sources have one degree of freedom with 16 residual degrees of freedom for each linear model.

| Source | DF | Nutrient | | Fish | | Fish × Nutrient | |
|---|---|---|---|---|---|---|---|
| | | F | p | F | p | F | p |
| AFDM (g × cm$^{-2}$) | 1,16 | **5.0** | **0.040** | *3.8* | *0.068* | 0.5 | 0.494 |
| Chl *a* (µg × cm$^{-2}$) | 1,16 | **26.1** | **0.001** | *3.3* | *0.086* | 0.1 | 0.726 |
| AI (×10$^3$) | 1,16 | **29.1** | **0.001** | 2.5 | 0.133 | 1.9 | 0.189 |
| C:N (molar) | 1,16 | **16.0** | **0.001** | **16.8** | **0.001** | **9.4** | **0.007** |
| Night CPUE | 1,16 | 1.3 | 0.279 | **7.2** | **0.016** | *4.2* | *0.056* |
| Migration | 1,16 | 0.3 | 0.620 | **17.7** | **0.001** | 0.1 | 0.823 |

The biomass and composition of the epilithon were affected by nutrients. ANOVA of linear models (Table 2, Fig. 2) indicates significant nutrient effects on all four epilithon measures. $NH_4^+$ was positively associated with AFDM and chl *a*, indicating that nutrients positively affected the biomass of the epilithon. The significant positive relationship between $NH_4^+$ and AI indicates that the epilithon was composed of a larger fraction of autotrophic cells in higher nutrient ponds. The nutrient content of the epilithon was also affected by nutrients, with the C:N of the epilithon negatively related to $NH_4^+$.

*Halocaridina rubra* abundance and body length, but not migration or weight, were affected by nutrients. ANOVA of a linear model of CPUE (square-root transformed; adjusted $r^2$ = 0.58; Fig. 2e–f) indicates that nutrients marginally significantly increased *H. rubra* abundance (F = 3.02, p = 0.09) and *H. rubra* were significantly longer in higher nutrient ponds (F = 7.72, p = 0.02). ANOVA of a binomially distributed generalized linear model of *H. rubra* migration (Table 2, Fig. 2g), however, indicates that migration was not affected by nutrients (F = 0.19, p = 0.67). Average weight was not affected by nutrients (F = 0.21, p = 0.65).

### Top–down effects

Fish effects on epilithon biomass and composition were parallel to nutrient effects (Table 2, Fig. 2). The presence of fish marginally significantly increased the biomass of chl *a* and AFDM in models with $NH_4^+$ (these effects were significant in models with SRP and DIN, Supplementary material Appendix A1 Table A1). Fish presence also increased the biomass of autotrophic cells relative to total epilithon biomass (AI). This effect was not significant in a model with $NH_4^+$ (p < 0.15), but it was significant in models with SRP and DIN (Supplementary material Appendix A1 Table A1). Fish effects on epilithon nutrient content were present both as an overall fish effect, with fish ponds being significantly lower in C:N than ponds without fish, and as a fish × nutrient interaction effect, with the negative effect of $NH_4^+$ on C:N significantly muted in ponds with fish (Fig. 2d).

Fish and time of day affected the counts of *H. rubra*. ANOVA of a linear model of CPUE (square-root transformed; adjusted $r^2$ = 0.58; Fig. 2e–f) indicates fish significantly reduced the abundance of *H. rubra* overall
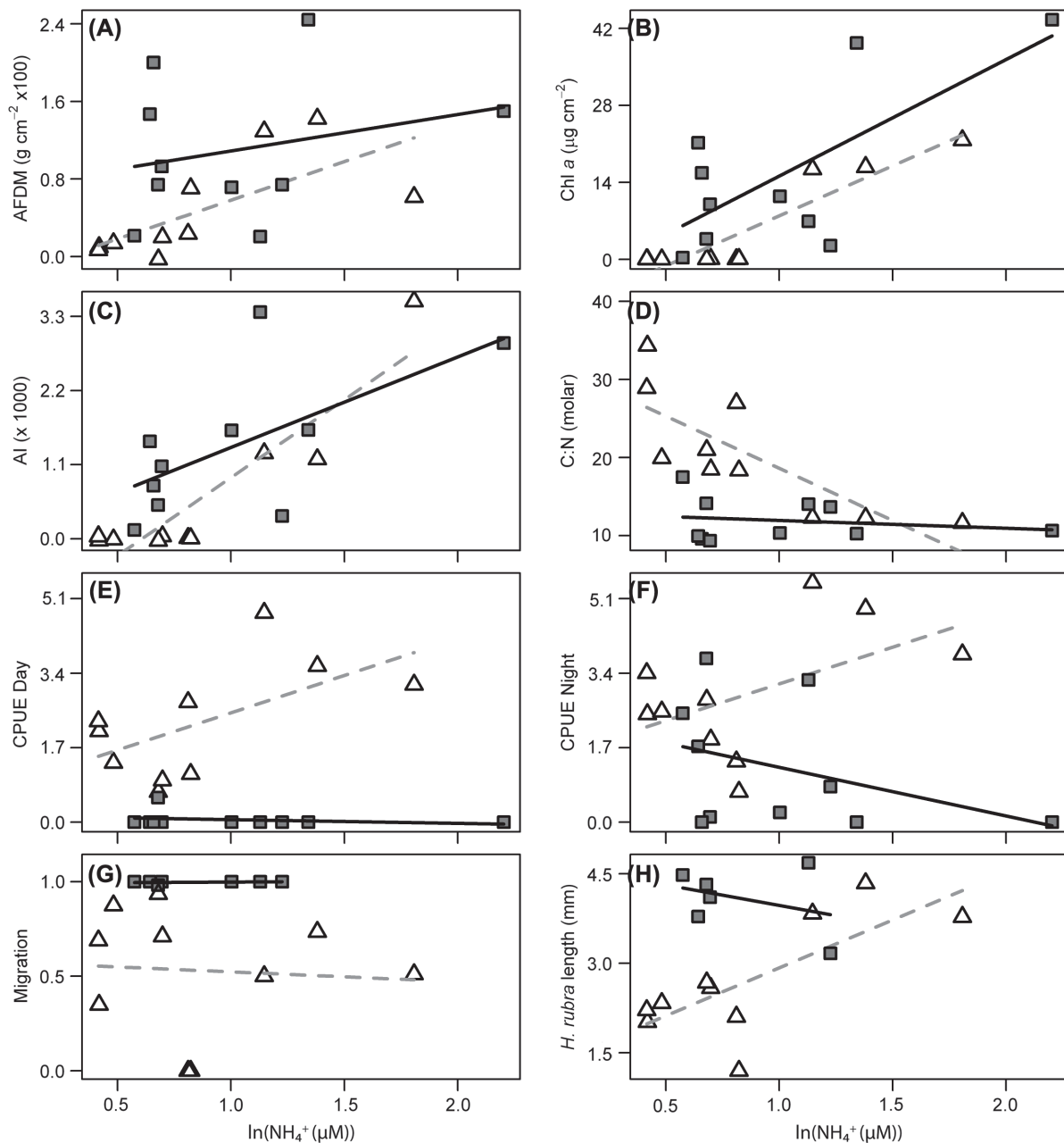
Figure 2. Food web measurements and associated linear relationships with log-transformed $NH_4^+$ concentration in fish ponds (filled gray squares, solid black line) and no-fish ponds (open triangles, dashed gray line) for (A) AFDM per area ($100 \times$ g cm$^{-2}$), (B) chl *a* per area (μg cm$^{-2}$), (C) autotrophic index (AI): chl *a* per AFDM ($\times 10^3$), (D) epilithon C:N (molar ratio), (E) *Halocaridina rubra* daytime abundance (CPUE), (F) *H. rubra* nighttime abundance (CPUE), (G) *H. rubra* migration (proportion of night population that leaves pond during the day), and (H) *H. rubra* length (mm). Lines are best fit results from linear models with log transformed $NH_4^+$ and presence of fish as factors (Table 2). Ponds where no *H. rubra* were captured have truncated regression for migration and *H. rubra* length, since neither metric applies to ponds with no *H. rubra*.

($F = 33.85$, $p = 1.8 \times 10^{-6}$) and eliminated the positive effect of nutrients on *H. rubra* abundance (fish $\times$ nutrient interaction effect: $F = 14.6$, $p = 0.001$). *Halocaridina rubra* counts were higher at night than during the day ($F = 7.22$, $p = 0.01$). These effects were confirmed in an analysis of individual net counts using a generalized linear mixed model for *H. rubra* net counts that corrected for effort using the 'weights' function (Crawley 2005), assumed counts were Poisson-distributed, and used pond as a random factor.

ANOVA of a binomially-distributed generalized linear model of migration indicates the presence of fish significantly increased the migration of *H. rubra* ($F = 11.7$, $p = 0.005$). In fish ponds, CPUE of *H. rubra* was 99% lower during the day, while, in no-fish ponds, CPUE of *H. rubra* was only 45% lower during the day. ANOVA of a linear model indicated *H. rubra* weights and lengths (Fig. 2h) were significantly larger in ponds with fish ($F = 7.05$, $p = 0.02$ for weight, $F = 21.49$, $p = 0.001$ for
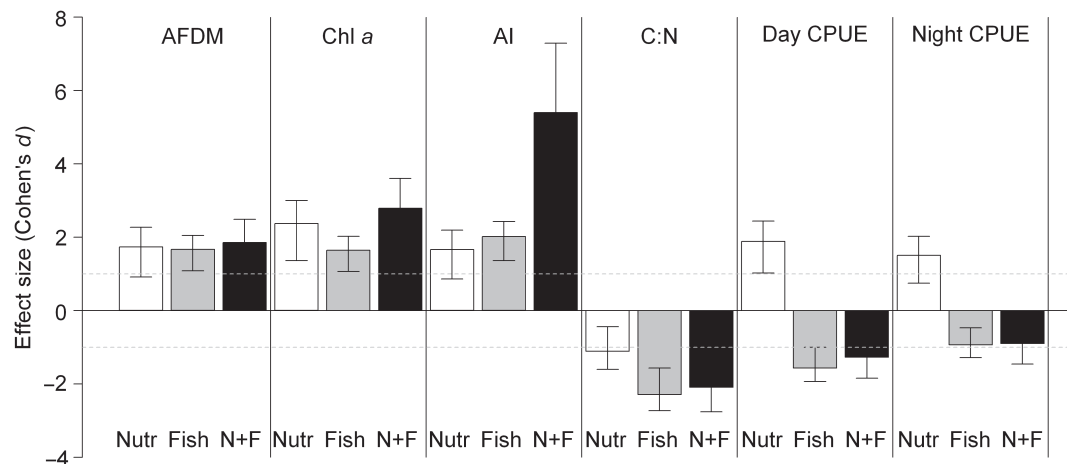
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Figure 3. Effect size (Cohen's *d*) of nutrients ('Nutr'), fish ('Fish'), and fish + nutrients ('N + F') on AFDM, chl *a*, AI, C:N, and *H. rubra* abundance at day and night. Error bars are standard errors (Nakagawa and Cuthill 2007). Dotted gray lines indicate a Cohen's *d* equal to one, often considered an indicator of a particularly strong effect size.

length) than in ponds without fish. Average weight was not affected by nutrients or fish × nutrient interactions (F = 0.62, p = 0.44 for fish × nutrient), but *H. rubra* was significantly longer in higher nutrient ponds (F = 7.72, p = 0.02).

## Contrasting bottom–up and top–down effects

The effect size of bottom–up and top–down influences on the epilithon were comparable (Fig. 3, Supplementary material Appendix A1 Table A3). Fish and nutrients increased AFDM and chl *a* by similar effect sizes, but the combined effect of fish and nutrients on either AFDM or chl *a* was only slightly greater than the effect of either acting alone. While ANOVA on linear models did not indicate a significant interaction between fish and nutrient effects, effect size measures suggest fish and nutrient effects may be less than strictly additive. In contrast, while fish and nutrients had similar positive effect sizes on AI, the combined effect of fish and nutrients on AI appears

at least additive, as is expected from ANOVA of models with SRP and DIN (Supplementary material Appendix A1 Table A2). Both fish and nutrients reduced C:N, with the effect of fish being approximately twice that of nutrients. Notably, the combined effect of fish and nutrients was very similar to the effect of fish acting alone, suggestive of the interaction between fish and nutrient effects highlighted by model selection and ANOVA.

Fish effects on the epilithon were presumably the result of reduced grazing from *H. rubra* due either to increased migration out of ponds during the day (trait-mediated indirect effect) or to reduced abundance of *H. rubra* at all times of day (density-mediated indirect effect). We used linear models with separate nutrient ($NH_4^+$), behavior (*H. rubra* migration), and abundance (maximum CPUE of *H. rubra*) effects to assess the strength of trait and density mediated effects on the epilithon (Table 3). The variance in the biomass of epilithon (AFDM) was better predicted by a model with only nutrient and abundance effects, but the biomass of photosynthesizing cells (chlorophyll *a*)

Table 3. Linear model selection based on corrected Akaike information criterion (AICc) decomposing fish effects into density-mediated (CPUE) and trait-mediated (Migration) effects on epilithon variables, after accounting for nutrient effects ($NH_4^+$ is $\ln(NH_4^+)$).

| | $r^2$ | Adj $r^2$ | AICc | ΔAICc | Rel. like | $w_i$ |
|---|---|---|---|---|---|---|
| Models for AFDM (g cm$^{-2}$) | | | | | | |
| $NH_4^+$ + CPUE | 0.25 | 0.14 | −127.4 | 0.0 | 1.00 | 0.56 |
| $NH_4^+$ + Migration | 0.20 | 0.09 | −126.3 | 1.1 | 0.59 | 0.33 |
| $NH_4^+$ + CPUE + Migration | 0.29 | 0.12 | −124.0 | 3.3 | 0.19 | 0.11 |
| Model for Chl *a* (mg cm$^{-2}$) | | | | | | |
| $NH_4^+$ + CPUE | 0.46 | 0.39 | 119.0 | 0.0 | 1.00 | 0.42 |
| $NH_4^+$ + Migration | 0.46 | 0.38 | 119.1 | 0.0 | 0.98 | 0.41 |
| $NH_4^+$ + CPUE + Migration | 0.53 | 0.42 | 120.9 | 1.9 | 0.38 | 0.16 |
| Model for AI | | | | | | |
| $NH_4^+$ + Migration | 0.59 | 0.53 | 282.5 | 0.0 | 1.00 | 0.69 |
| $NH_4^+$ + CPUE | 0.53 | 0.47 | 284.8 | 2.2 | 0.33 | 0.22 |
| $NH_4^+$ + CPUE + Migration | 0.59 | 0.50 | 286.6 | 4.1 | 0.13 | 0.09 |
| Model for C:N (molar) | | | | | | |
| $NH_4^+$ + Migration | 0.61 | 0.55 | 109.5 | 0.0 | 1.00 | 0.88 |
| $NH_4^+$ + CPUE + Migration | 0.61 | 0.52 | 113.6 | 4.1 | 0.13 | 0.11 |
| $NH_4^+$ + CPUE | 0.33 | 0.23 | 118.8 | 9.3 | 0.01 | 0.01 |

was at least as well predicted by a model with only migration and nutrient effects. The composition of the epilithon, both in terms of relative autotrophy (AI) and nutrient content (CN), was better predicted by models with only nutrient and migration terms, indicating that trait-mediated top–down control was more important than density-mediated top–down control for the composition of the epilithon.

Effect size estimates indicated that nutrients positively impacted *H. rubra* abundance at day and at night (Fig. 3), fish negatively impacted *H. rubra* abundance during day and at night, and nutrients did not offset the negative influence of fish on *H. rubra*. Effect sizes for *H. rubra* length and weight were not calculated because a sufficiently large sample of *H. rubra* could not be obtained in any of the high nutrient fish ponds.

# Discussion

Both top–down and bottom–up factors influence food webs in Hawaiian anchialine ponds, and anthropogenic disturbance of both factors alters these endemic ecosystems. Nutrients increase the quantity, relative autotrophy and nutrient content of the epilithon while increasing the size and abundance of the dominant consumer, *Halocaridina rubra*. Fish reduce the abundance of *H. rubra* and induce increased diel *H. rubra* migrations. Reduced abundance and increased migration of *H. rubra* release the epilithon from grazing, resulting in increased abundance, autotrophy and nutrient content of the epilithon in ponds with fish. Top–down effects transmitted by changed *H. rubra* behavior alter the composition of the epilithon, whereas top–down effects transmitted by changed *H. rubra* abundance affect the overall biomass of the epilithon. These results reveal that both bottom–up and top–down factors structure anchialine pond ecosystems, but trait-mediated top–down control causes qualitatively different changes to pond food webs than traditionally considered density-mediated effects. Bottom–up effects of fertilizer and wastewater run-off and top–down effects of introduced fish predators represent threats to these globally rare ecosystems.

## Bottom–up effects

In the absence of fish, nutrients increased the quantity (Fig. 2a–b), relative autotrophy (Fig. 2c) and quality (Fig. 2d) of the epilithon. These effects extended upward to the primary grazer, *H. rubra*, which was more abundant (Fig. 2e–f) and larger (Fig. 2h) in ponds with higher nutrients. The increase in standing stocks of primary producers and consumers likely reflects increased benthic productivity in the presence of higher nutrient concentrations, a conclusion supported by the increased area-specific (Fig. 2b) and biomass-specific (Fig. 2c) concentrations of chl *a* in high nutrient ponds (Fig. 2b–c; Morin et al. 1999).

Though *H. rubra* is considered a keystone species that maintains a diverse epilithon in Hawaiian anchialine ponds (Bailey-Brock and Brock 1993), our data indicate

that nutrient enrichment overwhelms *H. rubra* grazing by changing the epilithon. This change may occur because algal and cyanobacterial species favored under nutrient enrichment are less palatable (Irigoien 2005, Mitra and Flynn 2007) or because there is a substantial time lag between increased production under nutrient enrichment and increased *H. rubra* grazing (Irigoien 2005). Though we do not have direct evidence to support our suggestion of changed algal and cyanobacterial taxonomic composition under nutrient enrichment, the alternative, time lag hypothesis seems particularly unlikely given that nutrient enrichment began at least 15 years before the onset of this study.

## Top–down effects

Introduced poeciliids exert top–down control on anchialine pond food webs by altering the abundance and behavior of *H. rubra*, and the strength of this top–down effect is equal to or greater than the bottom–up nutrient effect (Fig. 3). In the presence of fish, *H. rubra* was less abundant (Fig. 2e), even at night (Fig. 2f), and a larger proportion migrated out of epigeal habitat during the day (Fig. 2h). Heightened migration is likely a behavioral response to predation risk, as poeciliids are visual predators that readily consume *H. rubra* (Sakihara unpubl., Capps et al. 2009, Carey et al. 2010). Reduced *H. rubra* abundance and increased migration affect the epilithon by increasing its quantity, productivity and nutrient content at least as much as nutrient enrichment (Fig. 2a–d, 3). Poeciliids are known to drive such cascades in more diverse systems (Hurlbert et al. 1972, Nagdali and Gupta 2002, Ho et al. 2011), but our study describes a trophic cascade whereby poeciliids alter entire pond ecosystems by reducing the abundance and feeding of a single species, *H. rubra*. The relative lack of other species of invertebrate grazers in this system, and, specifically, the lack of any species resistant to poeciliid predation, prevents compensation by other members of the community from offsetting the effects of reduced *H. rubra* grazing.

The top–down effects of predators in this system vary depending on whether those effects are transmitted by changes in the abundance or behavioral traits of *H. rubra*. Changes in the abundance of *H. rubra* altered the biomass of the epilithon, whereas changes in *H. rubra* migration altered in the epilithon composition (Table 3). Trait-driven effects may differ from density-driven effects because increased migration not only reduces the amount of grazing by *H. rubra* but also shifts when that grazing occurs. In the presence of fish, *H. rubra* predominately graze at night, providing a diurnal refuge for algal and cyanobacterial species in the epilithon and altering how the consumed nutrients are recycled within the ecosystem (Haupt et al. 2009), potentially changing dynamics among taxa. Evidence to support this suggestion comes from statistical analysis of AI. After accounting for any effects of nutrients and *H. rubra* abundance on AI, increased migration positively influenced AI ($t = 2.4$, $p = 0.03$), suggesting that migration affected the composition (i.e. proportion of autotrophic species) of the epilithon independent of the effects of *H. rubra* abundance.

## Contrasting top–down and bottom–up

The combined effects of nutrients and fish vary between epilithon and *H. rubra*. Though fish eliminate and may even reverse the positive effects of nutrients on *H. rubra* abundance, these interaction effects do not cascade down to the epilithon, which is generally best modeled with independent fish and nutrient effects that act in the same direction. Release from grazing pressure thus has the same effect on epilithon regardless of the ambient nutrient concentrations. The exception to this rule is epilithon nutrient content, which is influenced by fish, nutrient and fish × nutrient interaction effects.

Changes in the epilithon stoichiometry could result from several different mechanisms, with different implications for food webs. Nutrient- or grazing-driven changes in the epilithon stoichiometry can occur due to competitive replacement by new species of primary producers with altered stoichiometry (Liess and Kahlert 2009) or shifts towards more detritus in the epilithon (Hunter 1980). The flexible stoichiometry of primary producers, moreover, allows for changes in epilithon nutrient content without taxonomic shifts if grazing or nutrient enrichment reduce competition for limiting nutrients (McCormick and Stevenson 1991) or investment in chemical or structural defense (DeMott et al. 2010). Each mechanism of change in epilithon stoichiometry could have different implications for its bottom–up effect on the food web. Our data suggest that the proportion of the epilithon made up of photosynthesizing cells may largely affect C:N, as a model for C:N with only AI is a better predictor of epilithon C:N (AICc = 130.0) than models with only nutrients (AICc = 137.6) , presence of fish (AICc = 132.0), or a combination of the two factors (AICc = 131.3). More data on the taxonomic composition of the epilithon, however, would be necessary to determine what underlies the observed shift in the C:N of the epilithon, and how these changes in epilithon composition might travel up the food web.

In total, we assessed anchialine pond food webs by contrasting the stimulating effects of nutrient enrichment on primary productivity against the consumptive effects of additional trophic levels at the apex of the food web. This relatively simple perspective necessarily neglects complexity that may be important in this system. For example, ecosystem engineering activities by grazing *H. rubra* (grazing algae and cyanobacteria from rock crevices) may maintain shelter habitat that enables their persistence despite the presence of predatory fish. Reduced epilithon accumulation due to *H. rubra* grazing, moreover, may be especially important in this system for its role in maintaining groundwater and tidal flushing through the porous substrate. Studies in other systems have also linked poeciliid ecosystem effects to changes in nutrient cycles (Hurlbert et al. 1972, Nagdali and Gupta 2002, MacKenzie and Bruland 2011). Here, fish were only related to changes in the concentration of DIN, but the increased productivity of ponds with fish may result in higher rates of nutrient uptake by the epilithon (Carpenter and Dunham 1985), hindering our ability to detect fish effects on nutrient cycling based on concentrations of inorganic nutrients alone.

Additionally, in this study we have focused broadly on 'nutrient effects', but the apparent differences between the dynamics of $NH_4^+$ or SRP and DIN suggest each of these nutrients may place different controls on anchialine ecosystems. These controls, moreover, may be strongly influenced over time by temporal variation in the supply of nutrients to the ponds, especially in developed areas where short term human activities, especially management activities, can drive substantial change in nutrient concentrations (D. Chai, pers. comm.). Further study of the complex ecosystem engineering and other indirect effects in these anchialine ponds could elucidate the role such mechanisms play in maintaining food web structure in low diversity, highly endemic ecosystems.

## Implications for conservation

Human modification of bottom–up and top–down influences on anchialine pond food webs threatens to undermine the functioning of these globally rare ecosystems and their endemic flora and fauna. The nearly 600 anchialine ponds on the island of Hawai'i represent one of the world's largest concentrations of anchialine ponds (Wiegner et al. 2006) and contain unique invertebrate communities composed of both hypogeal and epigeal mollusks, insects and crustaceans. Several of these species are candidates for protection under the United States Endangered Species Act (USGS 2005).

Our results show that the effects of both nutrient loading and invasive fish have substantial consequences for anchialine ponds by altering the role that a single species, *H. rubra*, plays in maintaining the epilithon. The anchialine ponds of Hawai'i may be especially sensitive to changed environmental and food web factors because they have relatively low species diversity. Though many species of invertebrates have been recorded in Hawai'i's anchialine ponds, we observed only a few individuals of these species other than *H. rubra*, vastly fewer than the hundreds or thousands of individual *H. rubra* observed in nearly all of the ponds we visited.

Because of the important role *H. rubra* plays in maintaining the food web structure of anchialine ponds, it is important to consider the factors affecting its abundance. Fish have a marked effect on the abundance and activity of *H. rubra*, but total fish effects may extend beyond those observed in this study. For instance, the increased size of *H. rubra* in fish ponds, putting the animals outside the gape limitation of these small fish, may be the result of compensatory growth during periods when *H. rubra* is vulnerable to predation. Such compensatory growth could have negative consequences for *H. rubra* reproduction and population dynamics (Dmitriew and Rowe 2005, Auer 2010), though such effects in these populations are unknown. Nutrients, in contrast, positively affect *H. rubra* abundance in fishless ponds, but have very different effects in ponds with fish. The positive effects of nutrients were reversed in the presence of fish, with *H. rubra* extirpated from the highest nutrient fish ponds (Fig. 3). This change may occur because enrichment stimulates the productivity of epilithon that, without *H. rubra* grazing, accumulates to as much as 20 cm deep. This algal

mat can prevent *H. rubra* from accessing refuges in rock crevices and can increase their exposure to fish predation. Consumers are generally hindered by eutrophication-induced change in habitat structure (Diehl 1988, 1993, Manatunge et al. 2000), and, here, physical change in benthic environments from increased nutrients and reduced grazing likely interferes with foraging and refuge use by *H. rubra*, perhaps exacerbating already dramatic changes to the epilithon structure.

The anchialine ponds of Hawai'i are a hotbed of endemic and native animals and a topic of recent interest for understanding evolutionary patterns (Santos 2006, Craft et al. 2008, Russ et al. 2009). Anchialine ponds in Hawai'i face threats from a range of invaders (tilapia, *Macrobrachium lar*, *B. marinus*), whose role in driving food web change remains to be characterized. In this study we describe the fundamentally different threats to these ponds posed by nutrient enrichment and invasive fish and the non-additive response that occurs when both stressors occur together.

# References

Akaike, H. 1974. A new look at the statistical model identification. – IEEE Trans. Automatic Control 19: 716–723.

Anholt, B. and Werner, E. E. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. – Ecology 76: 2230–2234.

Anholt, B. and Werner, E. 1998. Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. – Evol. Ecol. 12: 729–738.

Auer, S. K. 2010. Phenotypic plasticity in adult life-history strategies compensates for a poor start in life in Trinidadian guppies (*Poecilia reticulata*). – Am. Nat. 176: 818–829.

Bailey-Brock, J. H. and Brock, R. E. 1993. Feeding, reproduction, and sense organs of the Hawaiian anchialine shrimp *Halocaridina rubra* (Atyidae). – Pacific Sci. 47: 338–355.

Brock, R. E. et al. 1987. Characteristics of water quality in anchialine ponds of Kona, Hawaii Coast. – Pacific Sci. 41: 200–208.

Capps, K. A. et al. 2009. Behavioral responses of the endemic shrimp *Halocaridina rubra* (Malacostraca: Atyidae) to an introduced fish, *Gambusia affinis* (Actinopterygii: Poeciliidae) and implications for the trophic structure of Hawaiian anchialine ponds. – Pacific Sci. 63: 27–38.

Carey, C. C. et al. 2010. Predator-dependent diel migration by *Halocaridina rubra* shrimp (Malacostraca: Atyidae) in Hawaiian anchialine pools. – Aquat. Ecol. 45: 35–41.

Carpenter, E. J. and Dunham, S. 1985. Nitrogenous nutrient uptake, primary production, and species composition of phytoplankton in the Carmans River Estuary, Long Island, New York. – Oecologia 30: 513–526.

Carpenter, S. et al. 1996. Chlorophyll variability, nutrient input, and grazing: evidence from whole-lake experiments. – Ecology 77: 725–735.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences, 2nd edn. – Routledge Academic.

Covich, A. P. et al. 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. – BioScience 54: 767.

Craft, J. et al. 2008. Islands under islands: the phylogeography and evolution of *Halocaridina rubra* Holthuis, 1963 (Crustacean: Decapoda: Atyidae) in the Hawaiian archipelago. – Limnol. Oceanogr. 53: 675–689.

Crawley, M. J. 2005. Statistics: an introduction using R. – Wiley.

Cross, W. F. et al. 2006. Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. – Ecology 87: 1556–1565.

DeMott, W. R. et al. 2010. Ontogeny of digestion in *Daphnia*: implications for the effectiveness of algal defenses. – Ecology 91: 540–548.

Diehl, S. 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. – Oikos 53: 207–214.

Diehl, S. 1993. Effects of habitat structure on resource availability, diet and growth of benthivorous perch, *Perca fluviatillis*. – Oikos 67: 403–414.

Dmitriew, C. and Rowe, L. 2005. Resource limitation, predation risk and compensatory growth in a damselfly. – Oecologia 142: 150–154.

Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. – Oikos 99: 201–219.

Friederichs, S. J. et al. 2011. Total phosphorus and piscivore mass as drivers of food web characteristics in shallow lakes. – Oikos 120: 756–765.

Gilliam, J. F. et al. 1989. Strong effects of foraging minnows on a stream benthic invertebrate community. – Ecology 70: 445–452.

Gruner, D. S. 2004. Attenuation of top–down and bottom–up forces in a complex terrestrial community. – Ecology 85: 3010–3022.

Hambright, K. D. et al. 2007. Grazer control of nitrogen fixation: phytoplankton taxonomic composition and ecosystem functioning. – Fundam. Appl. Limnol. Arch. Hydrobiolo. 170: 103–124.

Haupt, F. et al. 2009. *Daphnia* diel vertical migration: implications beyond zooplankton. – J. Plankton Res. 31: 515–524.

Hillebrand, H. and Kahlert, M. 2001. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. – Limnol. Oceanogr. 46: 1881–1898.

Ho, S. S. et al. 2011. Comparing food-web impacts of a native invertebrate and an invasive fish as predators in small floodplain wetlands. – Mar. Freshwater Res. 62: 372–382.

Hoekman, D. 2011. Relative importance of top–down and bottom–up forces in food webs of Sarracenia pitcher communities at a northern and a southern site. – Oecologia 165: 1073–1082.

Holthuis, L. B. 1973. Caridean shrimps found in land-locked salt water pools at four Indo-West Pacific localities (Sinai Peninsula, Funafuti Atoll, Maui, and Hawaiian Islands), with the description of one new genus and four new species. – Zool. Verhandel. 128: 1–48.

Hunter, R. 1980. Effects of grazing on the quantity and quality of freshwater aufwuchs. – Hydrobiologia 69: 251–259.

Hurlbert, S. H. et al. 1972. Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. – Science 175: 639–641.

Irigoien, X. 2005. Phytoplankton blooms: a "loophole" in micro-zooplankton grazing impact? – J. Plankton Res. 27: 313–321.

Jeppesen, E. et al. 2000. Trophic structure in the pelagial of 25 shallow New Zealand lakes: changes along nutrient and fish gradients. – J. Plankton Res. 22: 951–968.

Jeppesen, E. et al. 2003. The impact of nutrient state and lake depth on top–down control in the pelagic zone of lakes: a study of 466 lakes from the temperate zone to the Arctic. – Ecosystems 6: 313–325.

Liess, A. and Kahlert, M. 2009. Gastropod grazers affect periphyton nutrient stoichiometry by changing benthic algal taxonomy and through differential nutrient uptake. – J. N. Am. Benthol. Soc. 28: 283–293.

Loreau, M. et al. 2002. Biodiversity and ecosystem functioning: synthesis and perspectives. – Oxford Univ. Press.

MacKenzie, R. A. and Bruland, G. L. 2011. Nekton communities in Hawaiian coastal wetlands: the distribution and abundance of introduced fish species. – Estuaries Coasts 35: 212–226.

Manatunge, J. et al. 2000. The influence of structural complexity on fish–zooplankton interactions: a study using artificial submerged macrophytes. – Environ. Biol. Fishe. 58: 425–438.

McCormick, P. and Stevenson, R. 1991. Grazer control of nutrient availability in the periphyton. – Oecologia 86: 287–291.

Mitra, A. and Flynn, K. J. 2007. Importance of interactions between food quality, quantity, and gut transit time on consumer feeding, growth and trophic dynamics. – Am. Nat. 169: 632–646.

Mittelbach, G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. – Ecology 62: 1370–1386.

Morin, A. et al. 1999. Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. – J. N. Am. Benthol. Soc. 18: 299–307.

Nagdali, S. S. and Gupta, P. 2002. Impact of mass mortality of a mosquito fish, *Gambusia affinison* the ecology of a fresh water eutrophic lake (Lake Naini Tal, India). – Hydrobiologia 468: 45–51.

Nakagawa, S. and Cuthill, I. C. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. – Biol. Rev. Camb. Phil. Soc. 82: 591–605.

Norlin, J. I. et al. 2005. Submerged macrophytes, zooplankton and the predominance of low- over high-chlorophyll states in western boreal, shallow-water wetlands. – Freshwater Biol. 50: 868–881.

Obernborfer, R. Y. et al. 1984. The effect of invertebrate predators on leaf litter processing in an alpine stream. – Ecology 65: 1325–1331.

Parkos, J. J. and Wahl, D. H. 2010. Influence of body size and prey type on the willingness of age-0 fish to forage under predation risk. – Trans. Am. Fish. Soc. 139: 969–975.

Pena, E. A. and Slate, E. H. 2006. Global validation of linear model assumptions. – J. Am. Stat. Ass. 101: 341–354.

Rosemond, A. D. and Mulholland, P. J. 1993. Top–down and bottom–up control of stream periphyton: effects of nutrients and herbivores. – Ecology 74: 1264–1280.

Russ, A. et al. 2010. Genetic population structure of an anchialine shrimp, *Metabetaeus lohena* (Crustacea: Alpheidae), in the Hawaiian Islands. – Rev. Biol. Trop. 58: 159–170.

Santos, S. R. 2006. Patterns of genetic connectivity among anchialine habitats: a case study of the endemic Hawaiian shrimp *Halocaridina rubra* on the island of Hawaii. – Mol. Ecol. 15: 2699–2718.

Schmitz, O. J. et al. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. – Ecol. Lett. 7: 153–163.

Slavik, K. et al. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. – Ecology 85: 939–954.

Steinman, A. D. and Lamberti, G. A. 1996. Biomass and pigments of benthic algae. – In: Hauer, F. R. and Lamberti, G. A. (eds), Methods in stream ecology. Academic Press, p. 297.

Stevenson, R. J. and Bahls, L. L. 1999. No title. – In: Barbour, M. T. et al. (eds), Rapid bioassessment protocols for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates and fish, 2nd edn. EPA 841-B-99-002 US Environ. Protection Agency, pp. 6.1–6.22.

Trussell, G. C. et al. 2006. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. – Ecol. Lett. 9: 1245–1252.

USGS 2005. Inventory of anchialine pools in Hawaii's National Parks.

Wallace, J. B. and Webster, J. R. 1996. The role of macroinvertebrates in stream ecosystem function. – Annu. Rev. Entomol. 41: 115–139.

Wayman, R. G. 1975. Limnology. – W. B. Saunders Co.

Weitzel, R. L. 1979. Periphyton measurements and applications. – In: Weitzel, R. L. (ed.), Methods and measurements of periphyton communities: a review. Am. Soc. Testing and Materials, pp. 3–33.

Werner, E. and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. – Ecology 84: 1083–1100.

Werner, E. E. and Peacor, S. D. 2006. Lethal and nonlethal predator effects on an herbivore guild mediated by system productivity. – Ecology 87: 347–361.

Wiegner, T. et al. 2006. A review of coastal monitoring data for developments in west Hawai'i. – County of Hawai'i, Hilo, HI, in press.

Yamamoto, M. N. and Tagawa, A. W. 2000. Hawai'i's native and exotic freshwater animals. – Mutual Publishing.

Zaret, T. and Suffern, J. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. – Limnol. Oceanogr. 21: 804–813.

Supplementary material (available as Appendix O20696 at < www.oikosoffice.lu.se/appendix >). Appendix A1.