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Empirical measurements of ammonium excretion in kelp forest fishes: Effects of body size, taxonomy and trophic guild

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ABSTRACT

Fishes and other consumers excrete metabolic waste products, including dissolved nutrients rich in nitrogen, which is an essential nutrient for primary production. Relatively little is known about the magnitude and variability of nutrients excreted by fishes in kelp forest ecosystems and whether consumer-derived nutrients are important for supporting kelp productivity. In this study, the supply of ammonium (NH_4^+) excreted by the dominant fishes (30 species representing ~85% of total fish biomass) was investigated on nearshore rocky reefs in California. Using rapid field incubations, the amount of excreted dissolved ammonium was measured as a function of body size (n = 460 individuals) and predictive models were developed relating mass to excretion rates at the family-level. Mass-specific excretion rates ranged from 0.08 to 3.45 μmol·g^{-1}·hr^{-1}, and per capita ammonium excretion rates ranged from 5.9 to 2765 μmol·individual·hr^{-1}. Ammonium excretion scaled with fish body mass to the ¾ power, as predicted by the metabolic theory of ecology; mass-specific excretion rates were higher in smaller fishes, but larger fishes contributed more ammonium per individual. When controlling for body size, ammonium excretion rates were greatest among surfperch (Embiotocidae), damselfish (Pomacentridae), and wrasses (Labridae), and the general trophic groups of planktivores and micro-carnivores. When body size differences were considered, the greatest mean excretion rates per individual were observed in larger-bodied species, such as California Sheephead (*Semicossyphus pulcher*) and Lingcod (*Ophiodon elongatus*). Empirical estimates of nutrient excretion by fishes, among the first measured in temperate kelp forests, were consistent with those in other aquatic systems. Ultimately, empirically derived excretion rates are the first step to quantifying the relative importance of consumers to nutrient cycling in kelp forest ecosystems.

1. Introduction

Complex multitrophic interactions are a central characteristic of ecosystem function worldwide. Since the 1960's, ecologists have questioned the factors that control ecosystem structure and productivity by asking if an ecosystem is driven by "top-down" factors (e.g., food-web interactions), "bottom-up" effects (e.g., resource limitation), or a combination of the two (Hairston et al., 1960; Slobodkin et al., 1967; Hunter and Price, 1992). While consumers have primarily been categorized as exerting a "top-down" effect on ecosystem structure via predation (Vanni and Layne, 1997; Pauly et al., 1998), consumers may also play a pivotal "bottom-up" role through the excretion of nitrogen and phosphorous into the environment (Redfield et al., 1963; Haines and Wheeler, 1978; Caron et al., 2000). The biotic processes of consumption and excretion are tightly coupled, and the importance of nutrients excreted by consumers to maintain productivity is widely recognized in many ecosystems, including terrestrial grasslands (McNaughton et al., 1988), temperate tidepools (Bracken and Nielsen, 2004), and open ocean environments (Hernández-León et al., 2008; Roman and McCarthy, 2010).

As a consumer, fishes are recognized as playing critical roles in structuring aquatic ecosystems; however, the potential role of fishes in supplying and recycling nutrients to support primary production is much less appreciated. Fishes excrete ammonium as a nitrogenous waste product, with the majority of ammonium being excreted at the gills, and smaller amounts through urine and feces (Smith, 1929; Wood, 1958; Sayer and Davenport, 1987). As a reduced form of nitrogen, ammonium is frequently utilized more readily than other forms of nitrogen by primary producers (Haines and Wheeler, 1978). Thus, fishes can generate biogeochemical hotspots of production at localized scales, such as when resident fishes feed and shelter within one biogenic habitat (Bray et al., 1986), aggregate over coral reefs (Meyer et al., 1983; Meyer and Schultz,

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1985), or aggregate within deep stream runs (McIntyre et al., 2008). Anthropogenic effects such as the introduction of invasive species (Capps and Flecker, 2013) and overharvesting that removes important predators (Taylor et al., 2006) can have profound impacts on nutrient cycling, which can influence biodiversity and ecosystem structure. The magnitude of fish-derived nutrients delivered to an ecosystem may be altered due to human activities, such as fishing and habitat fragmentation, resulting in shifts in fish abundance, biomass, and assemblage composition (Layman et al., 2011; Allgeier et al., 2014; Shantz et al., 2015; Peters et al., 2019). Despite the recognized importance of consumer-derived nutrients in other systems, surprisingly few studies have systematically explored how animal consumers may affect nutrient cycling in kelp forest ecosystems.

In kelp forest ecosystems, excretion of waste products by consumers is often overlooked as an important source of nutrients. Kelp forests are one of most productive ecosystems in the world (Graham et al., 2016), and it is often assumed that nitrate from coastal upwelling provides the bulk of the nitrogen requirements for kelps and other macroalgae (Zimmerman and Kremer, 1984). However, studies suggest that upwelling may provide only half of the nitrogen required by giant kelp (*Macrocystis pyrifera*) in southern California (Fram et al., 2008) and that localized ammonium may provide the missing nitrogen needed for growth during low nutrient periods (Brzezinski et al., 2013). Only a few studies have explored the role of consumers in kelp forests; Bray et al. (1986) reported that ammonium production by Blacksmith (*Chromis punctipinnis*) is elevated at night in crevices near the base of giant kelp and that kelp blades readily absorb ammonium during incubation experiments conducted in the lab. Subsequent research into community-level trends revealed that the rate of ammonium excreted by Blacksmith (250 μmol m^{-2} h^{-1}) was an order of magnitude greater than invertebrates and small crevice-dwelling fishes (30 μmol m^{-2} h^{-1}) (Bray et al., 1988). A study by Green and McFarland (1994) reported elevated ammonium concentrations as water masses passed through feeding Blacksmith schools, with ammonium increasing with school size. More recently, Peters et al. (2019) demonstrated that invertebrate consumers in southern California can excrete significant ammonium into the environment. Furthermore, excretion by the invertebrate assemblage was affected by heatwaves, disease, and protection from fishing inside marine reserves. Epiphytic species living on kelp blades, such as bryozoans, can also excrete ammonium in sufficient quantities to influence kelp growth (Hurd et al., 1994). Given the paucity of research that has previously been conducted, kelp forests offer an excellent opportunity to study the role of fishes as potential mediators of nutrient cycles.

Consumer-derived nutrient excretion has the potential to influence nutrient cycling in kelp forests and the productivity of foundation species, such as giant kelp (Brzezinski et al., 2013). However, because ammonium excretion has only been measured for a few fish species (Bray et al., 1988), the magnitude of nutrients excreted by the full kelp forest fish community is currently unknown. In addition, to quantify excretion at the community level, detailed information is required relating rates of ammonium excretion to factors such as body size, taxonomy, and feeding guild. In freshwater and tropical marine ecosystems, two conflicting theories have developed to predict the variables that best explain how individual demands for energy and nutrients influence excretion rates: the metabolic theory of ecology (MTE) and ecological stoichiometry (ES). The metabolic theory of ecology identified body size and temperature as key variables influencing variability in excretion among taxa (Brown et al., 2004). For most aquatic animals, excretion rates scale allometrically with body size, and larger taxa excrete ammonium at a lower rate per gram of biomass than smaller taxa (Wen and Peters, 1994; Brown et al., 2007; Vanni and McIntyre, 2016). Thus, it is predicted that total biomass being equal, an assemblage with small-bodied animals will have greater animal-mediated nutrient fluxes than an assemblage composed of large-bodied animals. Furthermore, under principles of the MTE, it is predicted that organisms within a particular taxonomic or functional group will excrete nutrients at similar rates due to relatively similar metabolic needs, whereas nutrient excretion will differ among disparate groups (Sterner and Elser, 2002; Vanni et al., 2002; Allgeier et al., 2015). The ecological stoichiometry theory predicts that elements within animals remain balanced with the environment (Reiners, 1986) and consumer excretion rates are affected by the nutrient content of their prey (Elser and Urabe, 1999; Sterner and Elser, 2002). Under principles of ES, trophic guild is predicted to explain some of the variation in excretion rates among taxa, as different prey types affect the quality of the food an individual consumes and how much it excretes (Schindler and Eby, 1997; Sterner and Elser, 2002; Vanni and McIntyre, 2016). Recent synthetic analyses have provided more support for the importance of MTE in explaining nutrient recycling across diverse groups of aquatic organisms (Allgeier et al., 2015; Vanni and McIntyre, 2016).

Kelp forests are dynamic systems that contain a high diversity of animal species, complex food webs, and trophic levels that are well-established in the literature (Hobson and Chess, 1986; Love et al., 2002; Graham, 2004). Our primary objective was to quantify ammonium excretion rates of the dominant fish species inhabiting kelp forests in central and southern California and evaluate the relative importance of body size, taxonomy, and trophic group. Our secondary objective was to aid methodological refinement by evaluating whether the timing and length of the experimental incubations influenced empirical estimates of ammonium excretion. We hypothesized that ammonium excretion rates will scale predictably with fish body mass across different taxa, such that excretion rates will increase with body size at similar rates, regardless of the magnitude of nutrients excreted. However, on top of the effect of body size we predicted that taxonomic identity and trophic guild will influence ammonium excretion due to phylogeny, dietary nutrient composition and quality, and prey digestibility. For example, we predicted that damselfish (family Pomacentridae) and surfperch (family Embiotocidae), which consume easily digestible zooplankton and small crustaceans continuously throughout the day, will excrete ammonium at faster rates for a given body size than families such as the chubs (family Kyphosidae), which consume a higher percentage of macroalgae.

2. Materials and methods

To quantify rates of ammonium excretion for the dominant fish species inhabiting nearshore rocky reefs and kelp beds in central and southern California, we utilized established techniques in the field for rapid empirical estimation of consumer-derived nutrient excretion in aquatic systems (Schaus et al., 1997; Layman et al., 2011; Vanni et al., 2002; Whiles et al., 2009; Allgeier et al., 2014; Peters et al., 2019). Studies have shown estimates in the field are comparable to those in more controlled laboratory settings (Mather et al., 1995). In addition, field estimates likely better reflect variability of nutrient excretion in the wild following consumption of natural prey and differential elapsed time since the consumption of the most recent meal (Schaus et al., 1997; Wood, 2001). During incubations, animals were separated from potential food resources because these resources can release or take up nutrients. Because excretion rates are lower in fasting animals (Wood, 2001; Whiles et al., 2009), incubations are typically short so that they can be assumed to reflect excretion rates of animals feeding and growing naturally. We were not interested in measuring basal excretion following fasting in controlled laboratory conditions, which is often abnormally low compared to fish in natural settings (Wood, 2001), or trying to simulate field conditions in the lab, but instead to further understand the range of variability in excretion in natural settings across species as a function of body size and trophic groupings.

We selected a subset of 30 species, from 13 families, to represent the dominant fish species comprising California kelp forests. The selected species represented ~85% of total fish biomass, based on estimates of density and biomass from extensive underwater visual surveys conducted by the Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO) in central and southern California (Malone et al., 2022). Each

fish species was assigned to one of five trophic guilds based on discrete, fixed trophic delineations (herbivore/omnivore, planktivore, micro-carnivore, macro-carnivore, and piscivore) as established in the literature (Hobson and Chess, 1986; Love, 2011). Herbivore/omnivores consist of species that consume a relatively large proportion of algae along with some animal matter, planktivores eat zooplankton, micro-carnivores eat small crustaceans (e.g., amphipods, isopods, shrimps, crabs, and copepods), macro-carnivores eat larger invertebrates (e.g., gastropods, crabs, polychaetes, shrimps, sea urchins) and may occasionally eat small fishes, and piscivores eat primarily fishes (Hobson and Chess, 1986; Love, 2011). Since many fishes within California kelp forests are opportunistic predators, species with a mixed diet were classified into a single trophic group based on the dominant type of prey taxa (>50% of the diet) consumed at the adult stage.

2.1. Fish collections

Collections occurred from May 2016 – September 2017. Kelp forest fish communities are not uniform across coastal California; Point Conception is a recognized geographic break and distinct species typically associate with kelp forests north or south of this break (Horn and Allen, 1978; Allen et al., 2006; Miller, 2023). In central and northern California, species that evolve in cool, temperate environments (e.g., rockfishes, greenlings, surfperch) are most abundant, whereas in southern California, species from subtropical eastern Pacific lineages (e.g., wrasses, damselfish, sea basses) are dominant (Allen et al., 2006; Miller, 2023). To quantify excretion rates for species representative of kelp forests along the entire coast of California, we sampled fish in both regions (Fig. S1). Species affiliated with colder water were collected from Stillwater Cove (36°33′42.8"N 121°56′48.5"W) and Point Lobos (36°31′18.4"N 121°56′33.9"W) in central California, whereas species affiliated with warmer water were collected off Santa Catalina Island (33°26′52.8"N 118°28′57.4"W) in southern California.

For each species, we collected $n = 5$–45 individuals across a range of body sizes via a variety of methods, including hook-and-line and baited fish traps, as well as barrier nets and hand nets while SCUBA diving. Previous studies indicated that ~20–30 individuals per species is sufficient to generate a strong relationship between body size and nutrient excretion rates (Vanni et al., 2002; Layman et al., 2011; Allgeier et al., 2014). Species with fewer than 5 individuals collected were pooled with similarly related species in the same family during initial stage of species-level data analysis (Table 1). Past studies have indicated that nitrogen excretion in fish is similar within a family, but differs among families (Vanni et al., 2002; Allgeier et al., 2015). Fish were collected during daylight hours between 0900 and 1500, and only individuals collected in good condition (i.e., not exhibiting signs of barotrauma) underwent experimental incubation to measure ammonium excretion rates. After capture, fish were either placed directly in a prepared incubation chamber or in a communal holding tank for <30 min, in cases where the fishing yield exceeded the rate of experimental processing. All containers with fish were covered and aerated to reduce stress. To reduce handling stress, we weighed each individual for wet mass (in grams) and measured total and standard length (in centimeters) following incubation. Fish were then released alive at the site of capture.

2.2. Experimental incubations to measure ammonium excretion

To calculate the ammonium excretion rate (NH_4^+ per individual per

Table 1
Mean ammonium excretion rates of common kelp forest fishes by species ranked by mean per capita excretion rates.

				Wet Mass		Mass-specific excretion[a]	Per capita excretion[b]
				(g per indiv.)		(μmol $NH_4^+ \cdot g^{-1} \cdot hr^{-1}$)	(μmol $NH_4^+ \cdot hr^{-1}$)
Species	**Common name**	**Family**	**n**	**Min**	**Max**	**Mean ± SE**	**Mean ± SE**
Ophiodon elongatus	Lingcod	Hexagrammidae	10	910	4670	0.63 ± 0.33	2765 ± 420
Other *Sebastes* spp.	Treefish, Bocaccio, Vermilion Rockfish (RF)	Sebastidae	8	250	2600	0.79 ± 0.45	1133 ± 436
Semicossyphus pulcher	California Sheephead	Labridae	56	34	3470	0.56 ± 0.07	965 ± 86
Myliobatis californica	Bat Ray	Myliobatidae	11	1100	17,600	0.42 ± 0.03	838 ± 105
Scorpaenichthys marmoratus	Cabezon	Cottidae	12	890	2250	0.50 ± 0.32	581 ± 111
Girella nigricans	Opaleye	Girellidae	21	295	705	0.71 ± 0.39	464 ± 40
Medialuna californiensis	Halfmoon	Kyphosidae	22	130	625	1.44 ± 0.34	417 ± 53
Sebastes auriculatus/caurinus	Brown RF, Copper RF	Sebastidae	9	200	2000	0.26 ± 0.09	410 ± 81
Paralabrax clathratus	Kelp Bass	Serranidae	44	19	2240	0.76 ± 0.07	374 ± 62
Hexagrammos decagrammus	Kelp Greenling	Hexagrammidae	4	460	630	1.39 ± 1.41	342 ± 96
Caulolatilus princeps	Ocean Whitefish	Malacanthidae	12	75	590	0.48 ± 0.30	297 ± 40
Embiotocidae spp.	Black, Striped, Rainbow, Pile surfperches	Embiotocidae	14	15	605	0.73 ± 0.27	287 ± 54
Hypsypops rubicundus	Garibaldi	Pomacentridae	28	23	470	0.42 ± 0.14	273 ± 28.2
Sebastes atrovirens	Kelp Rockfish	Sebastidae	14	0.3	670	0.47 ± 0.12	235 ± 28
Sebastes serranoides/ flavidus/ melanops	Olive, Yellowtail, Black rockfish	Sebastidae	16	1.4	1320	0.37 ± 0.10	256 ± 56
Sebastes chrysomelas	Black and yellow Rockfish	Sebastidae	11	330	500	0.70 ± 0.47	254 ± 27.1
Halichoeres semicinctus	Rock Wrasse	Labridae	43	5.8	204	1.90 ± 0.26	161 ± 15.2
Scorpaena guttata	California Scorpionfish	Scorpaendiae	16	14	840	0.48 ± 0.13	132 ± 40
Sebastes carnatus	Gopher Rockfish	Sebastidae	11	1.0	580	0.46 ± 0.13	130 ± 37.2
Brachyistius frenatus	Kelp Surfperch	Embiotocidae	10	4.0	75	3.45 ± 0.70	110 ± 28.5
Sebastes mystinus	Blue Rockfish	Sebastidae	12	2.7	510	0.65 ± 0.11	112 ± 42.7
Oxyjulis californica	Señorita	Labridae	13	11	55	3.18 ± 0.64	80 ± 18.1
Chromis punctipinnis	Blacksmith	Pomacentridae	36	0.4	85	1.63 ± 0.22	59 ± 7.6
Oxylebius pictus	Painted Greenling	Hexagrammidae	12	2.3	50	1.57 ± 0.46	43 ± 7.8
Rhinogobiops nicholsii	Blackeye Goby	Gobiidae	10	1.7	14	0.24 ± 0.51	9.3 ± 2.0
Alloclinus holderi	Island Kelpfish	Clinidae	5	4.9	21	0.08 ± 0.33	5.9 ± 1.8
	Total		**460**				

[a]Mass-specific excretion rates were calculated as total ammonium excretion divided by the wet weight (g) of an individual. [b]Per capita excretion rates were averaged over all sizes measured for that species. Species are arranged in order of decreasing per capita excretion rates. Sample size n = the number of incubated individuals. In the cases where the sample size of a specific species was not robust, individuals of closely-related species by taxonomic identity and trophic group were grouped together.

gram body weight per hour) for each individual fish, we conducted experimental incubations to test the change in ammonium over a set time period. Each tank was lined with a 4 mm polyethylene bag, and the bag was changed between incubations. The tanks were filled with a known volume of seawater (1–75 L) which varied according to the size of the fish. During data analysis, nutrient excretion rates were calculated by standardizing the measured nutrient concentration by the water volume used. Seawater was collected from the surface using a submersible pump and water temperatures were within 2 °C of the temperatures from which the fishes were collected. Seawater was filtered to 1 μM using a sediment cartridge polypropylene filter (ANSI/NSF Standard 8″ x 2.5″), which removed autotrophic bacteria and plankton that may otherwise have altered nutrient concentrations during the incubation period. Although some bacteria and plankton are smaller than 1 μM, it is unlikely that these organisms affected the nitrogen levels in the seawater during the experiment. Control incubations without fish were conducted ($n = 4$ per day) to quantify any changes in ammonium levels not due to excretion by the fish. While typically negligible, changes in ammonium levels in control tanks were factored into the calculated rates of ammonium excretion for fish run each day.

Given the limited studies of nutrient excretion in kelp forest fishes, we tested the effects of incubation time on ammonium excretion rate in numerous species to help set the required incubation duration. At the start of the experiment, we conducted experimental time trials on a subset of $n = 135$ of the 460 individuals (5–15 individuals of each species), representative of a suite of size classes. An initial sea water sample was collected to represent the concentration of ambient ammonium without the fish in the tank. For the time trials, 7 samples were collected per incubation in 10-min intervals for one hour to account for the potential initial elevation of excretion rates due to stress resulting from capture and handling and the potential decline in excretion due to fasting post-capture (Whiles et al., 2009). At each time point, 2.5 mL of seawater was collected with a syringe affixed with a 0.45-μm Whatman nylon membrane filter, then the filtered sample was placed into a 20 mL amber glass scintillation vial pre-filled with working reagent, and stored on ice for later nutrient analysis at the lab. These analyses indicated that the excretion rate did not vary significantly as a function of experimental duration for any fish family (see Figs. 1 and 2). Thus, we shortened the incubation duration to 30 min and collected three samples in 15-min intervals (e.g., initial without fish, 15 min, and 30 min) for the remainder of the $n = 325$ individuals we incubated. Previous research in tropical systems has also suggested that 30 min is sufficient to accurately quantify ammonium excretion in fishes (Allgeier et al., 2014).

2.3. Nutrient analysis

Samples for ammonium quantification underwent nutrient analysis

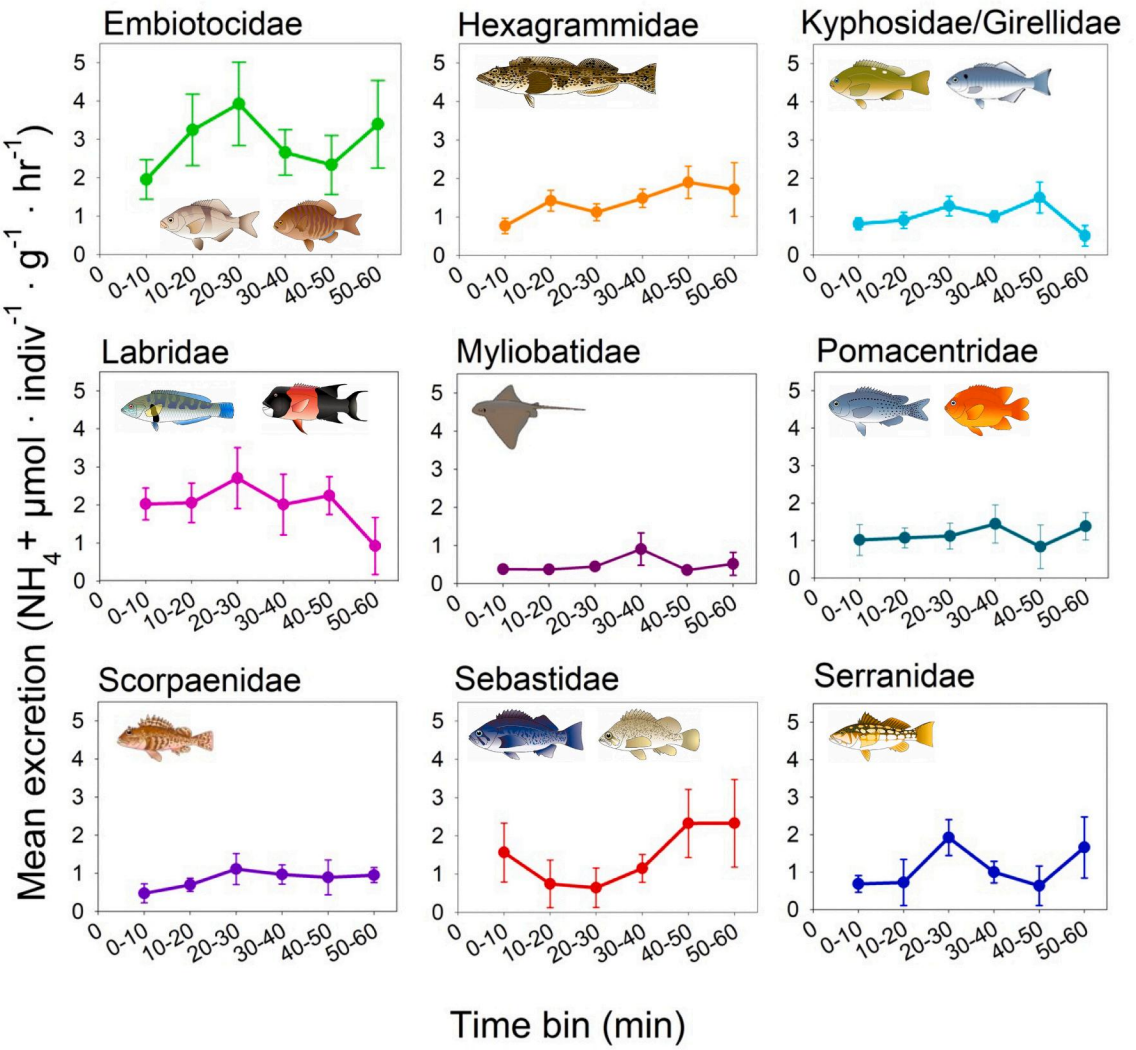

Fig. 1. Fish excretion rates over the duration of the experiment. Each point represents the mean (± 1 standard error) change in ammonium excretion per individual compared to the previous 10-min time bin for each family with representative species shown.

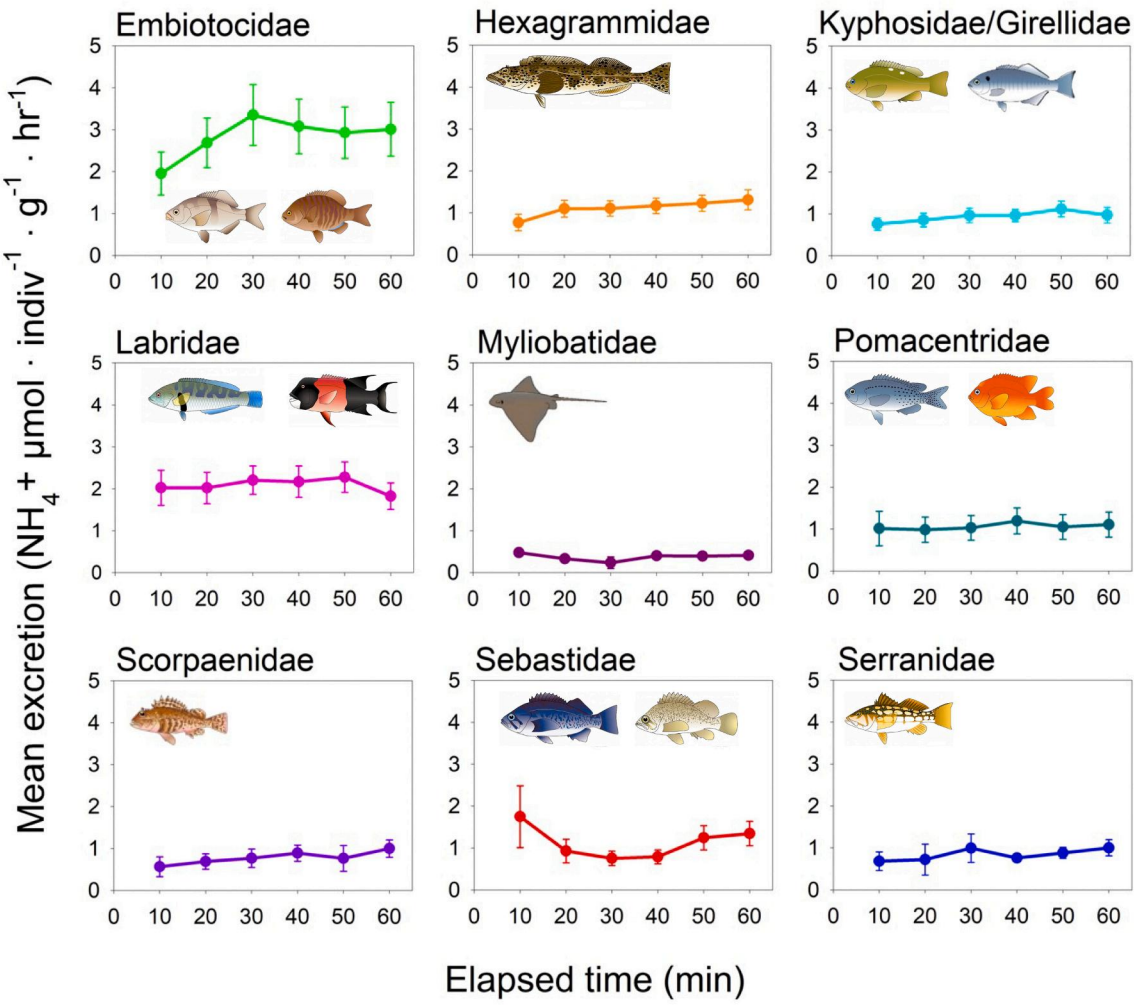

Fig. 2. Fish excretion rates at different incubation intervals. Each point represents the mean (± 1 standard error) change in ammonium excretion from the initial measurement at time = 0, per individual fish by family with representative species shown.

on the day of collection using established fluorometric techniques (Holmes et al., 1999, Protocol B). In the field, we added seawater samples to amber vials containing working reagent to begin the reaction process. The working reagent contained a mix of ortho-phthalaldehyde (OPA), sodium sulfite, and sodium tetraborate. Within 3–10 h of collection, we analyzed the ammonium samples using a handheld Turner Designs AquaFluor Handheld Fluorometer (optical kit No. 8000–402). Standards with known concentrations of ammonium were used daily to create a standard curve for calculating ammonium concentrations and underwent the same treatment in the field. Each morning, we created standards ranging from 0 to 40 µM with an ammonium stock solution using low nutrient seawater. Separate batches of low nutrient seawater were produced for each fish collection region in order to capture the background fluorescence in the water of that region.

2.4. Data analysis

To calculate the excretion rate of each individual, we plotted NH_4^+ (µmol) as a function of experimental duration (minutes) and extracted the slope of the relationship using linear regression. Mass-specific excretion rates by species were calculated as total ammonium excreted per individual per hour, divided by the wet weight (g) of an individual. Per capita excretion rates were averaged over all sizes measured for that species, and reflect the total ammonium excreted, regardless of body size. Species with similar taxonomic identities and/or feeding habits were later grouped together and excretion was calculated at the level of a fish family or trophic guild, respectively.

To test if the excretion rate varied throughout the duration of an hour-long incubation, in response to the initial stress of capture and handling, we calculated the change in ammonium excretion in ten-minute time bins (e.g., 0–10 min, 10–20 min, 20–30 min, etc.) for each fish family and used an ANOVA to test if excretion rates differed among time periods. Data were $\log_{10}$ transformed to meet assumptions of normality. We also tested the effect of experimental duration on the ammonium excretion rate over different cumulative time intervals to test whether excretion estimates improve with longer duration incubations. To determine if the excretion rate from the initial timepoint to differing endpoints stayed constant, we subtracted the mean mass-specific excretion rate at various secondary intervals (e.g., 10 min, 30 min, 60 min, etc.) from the initial ammonium concentration value without a fish. We then used an ANOVA to test if excretion depended on the length of the incubation duration for each fish family sampled. To test the effects of capture methods (hook and line, barrier and hand nets on SCUBA, or fish traps) on mass-specific ammonium excretion, we used those species that had $n > 5$ incubations for each method. Because most species were primarily caught with a single capture method, this limited the analysis to two species.

To account for the effect of fish mass on excretion rates at the species-, family-, and trophic group-levels, we used bivariate linear regressions to examine the strength of the association between NH_4^+ excretion per individual per minute as a function of fish mass. Due to evidence of allometric scaling for larger fish, we log-transformed excretion and body

size to improve homoscedasticity and normality. To test the hypothesis that the concentration of ammonium excreted by kelp forest fishes differs based on phylogeny, we used an Analysis of Covariance (ANCOVA) with the factor of family identity, the covariate of body mass, and the interaction between family identity and body mass. The families Cottidae, Gobiidae, and Malacanthidae were excluded due to limited sample sizes and/or a limited range of body sizes sampled. To test the hypothesis that the concentration of ammonium excreted by kelp forest fishes differs among trophic guilds, we used an ANCOVA to test for statistically significant differences in mass-specific excretion rates with the factor of trophic guilds, the covariate of body size, and the interaction between the two. We examined effects of time of day of capture on excretion rates within species, to control for potential effects of the time since the last meal, and did not detect any significant effects. All statistical analyses were conducted using JMP 14.

3. Results

3.1. Quantifying the effects of incubation duration and capture method on NH_4^+ excretion

Despite the variability in excretion rates among 10-min time bins for some families (Fig. 1), there was no statistically significant effect of time on excretion for any family over the experimental durations used in this study (Table S1A). In addition, there was not a significant effect of increasing incubation duration on ammonium excretion rates for any fish family (Fig. 2; Table S1B). Thus, conducting an experiment for 30 min yields the same excretion rate as an experiment for 60 min. These results also suggest that occasionally using a holding chamber for a short period (10–30 min) before starting the incubation did not significantly alter estimated excretion rates. Because ammonium excretion did not decline over time for any group or vary with incubation duration, we conclude that our techniques were unlikely to be influenced by initial stress of capture or fasting effects for the species and duration studied.

Two species, Kelp Bass (*Paralabrax clathratus*) and California Sheephead (*Semicossyphus pulcher*) had sufficient sample sizes ($n > 5$ per method) to evaluate the effects of capture method on ammonium excretion rates (Fig. 3). For both species, we did not detect any significant differences in excretion between fish captured using hook-and-line, underwater barrier nets on SCUBA, or fish traps (ANOVA: Kelp Bass, $F_{2,39} = 0.79$, $P = 0.46$; California Sheephead, $F_{2,55} = 0.39$, $P = 0.68$). Thus, excretion rates in our field incubations were equivalent regardless of the capture technique.

3.2. Effects of taxonomy on NH_4^+ excretion rates

Mass-specific and per capita rates of ammonium excretion varied significantly among the fish species sampled. The mean mass-specific excretion rate was 0.70 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ and ranged from 0.08 to 3.45 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ for different species (Table 1). Kelp Surfperch (*Brachyistius frenatus*) and Señorita (*Oxyjulis californica*) excreted ammonium at the highest rates per gram of body weight of the 30 species sampled (3.45 and 3.18 $\mu mol \cdot g^{-1} \cdot hr^{-1}$, respectively). However, when the collinear variable of size was allowed to propagate through, the average magnitude of ammonium excreted by an individual fish in an hour increased by 2–3 orders of magnitude (Table 1). Individual fish excretion rates ranged from 5.9 to 2765 $\mu mol\ NH_4^+ \cdot individual^{-1} \cdot hr^{-1}$, and the mean excretion rate was 412.1 ± 27.83. A large 4.6 kg Lingcod individual excreted the most ammonium (2765 $\mu mol \cdot hr^{-1}$), which was nearly two times higher than a 3.5 kg California Sheephead (965 $\mu mol \cdot hr^{-1}$). Smaller fishes, such as Island Kelpfish and Blackeye Goby (<5 g), excreted the least per individual at 5.9 and 9.3 $\mu mol\ NH_4^+ \cdot individual^{-1} \cdot hr^{-1}$, respectively.

Of the 460 incubations, per capita excretion rates scaled allometrically (Fig. 4). Overall, larger bodied fishes excreted more ammonium than smaller bodied fishes. However, the rate of ammonium excretion per gram of body mass was not equivalent and scaled with mass to a power of 0.75 across all fish individuals and species sampled (Fig. 5A). Across all families, nutrient excretion increased with mass (Fig. 5B), however the excretion rate differed significantly among families (Fig. 5C; ANCOVA, family: $F_{7,394} = 17.32$, $P < 0.0001$; body mass: $F_{1,394} = 569.5$, $P < 0.0001$). The interaction term was statistically significant, indicating that the slopes were not constant and excretion rates differed among families (ANCOVA, family*body mass: $F_{7,394} = 3.67$, $P = 0.0007$); although excretion was similar for all families at small sizes, excretion rates diverged as fish grew larger (Fig. 5B). For example, as body size increased, fishes in the family Labridae excreted nearly double the concentration of ammonium per hour compared to the same sized individuals in the Sebastidae and Scorpaenidae. Overall, the fit of the linear regressions indicated high predictive potential of fish excretion as a function of wet mass (r^2 values ranged from 0.34 to 0.94). Higher r^2 values often reflected families that were better sampled in the dataset (Fig. 4). For a full list of predictive ammonium excretion equations as a function of body mass for each family, see the Electronic Supplementary Information (Table S2).

Of the thirteen families tested, the mean mass-specific excretion rate ranged from 0.4 to 2.4 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ (Table 2). On average across all fish sizes, families differed in mean excretion rates per gram of fish mass (ANOVA: $F_{7,402} = 15.07$, $P < 0.0001$). Embiotocids, Pomacentrids, and Labrids on average excreted ammonium at higher rates per gram of body

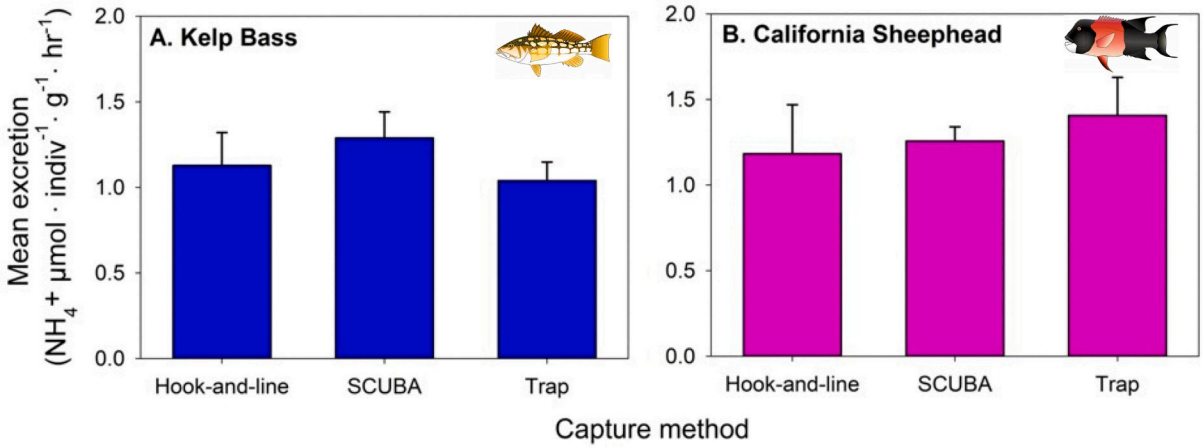

Fig. 3. Effects of capture method on ammonium excretion rates for (A) Kelp Bass and (B) California Sheephead. Shown are mean mass-specific excretion rates ±1 SE.

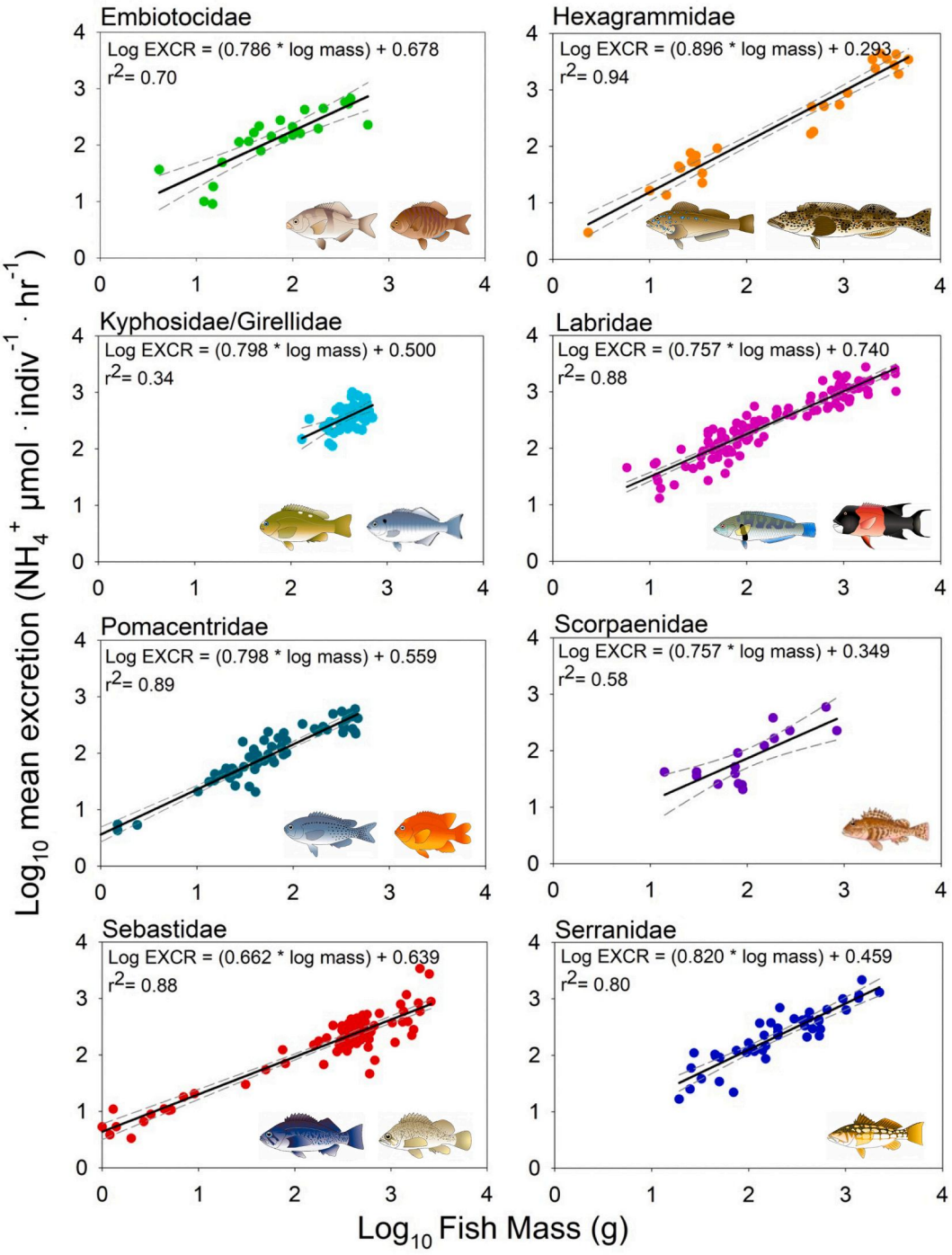

Fig. 4. Ammonium excretion rates as a function of body size for each fish family. All values were $\log_{10}$ transformed and each point represents an individual fish. Fish body weight is shown on the x-axis, and the excretion rate is shown on the y-axis. Solid line depicts the best fit linear equation, while the dashed curves indicated 95% confidence intervals on the best-fit line. Each panel represents a different family with representative species shown.

mass, excreting over two times more ammonium than individuals of the Serranidae, Kyphosidae, Scorpaenidae, and Sebastidae families (Fig. 5C; Table 2). However, when body size was considered, families with larger bodied individual fish (Hexagrammidae, Kyphosidae, Labridae, Serranidae) excreted ammonium 2–3 times faster than the smaller-bodied fish families that we incubated (Table 2).

3.3. *Effects of trophic group on NH_4^+ excretion rates*

There were significant differences in excretion among fish trophic groups, but the slopes of the relationships (i.e., rate of excretion as a function of body mass) were similar (Fig. 5D), as indicated by the non-significant interaction between trophic guild and body mass (ANCOVA, trophic guild: $F_{4,435} = 13.50$, $P < 0.0001$; body mass: $F_{1,435} = 246.0$, $P < 0.0001$; trophic guild*body mass: $F_{4,435} = 0.8603$, $P = 0.4877$). On average, mass-specific excretion rates were 2× greater among planktivores and micro-carnivores compared to macro-carnivores, piscivores, and herbivore/omnivores (ANOVA: $F_{4,440} = 36.72$, $P < 0.0001$; Table 3; Fig. 5E). However, on the per individual basis, larger-bodied macro-carnivores excreted an order of magnitude more ammonium than small-

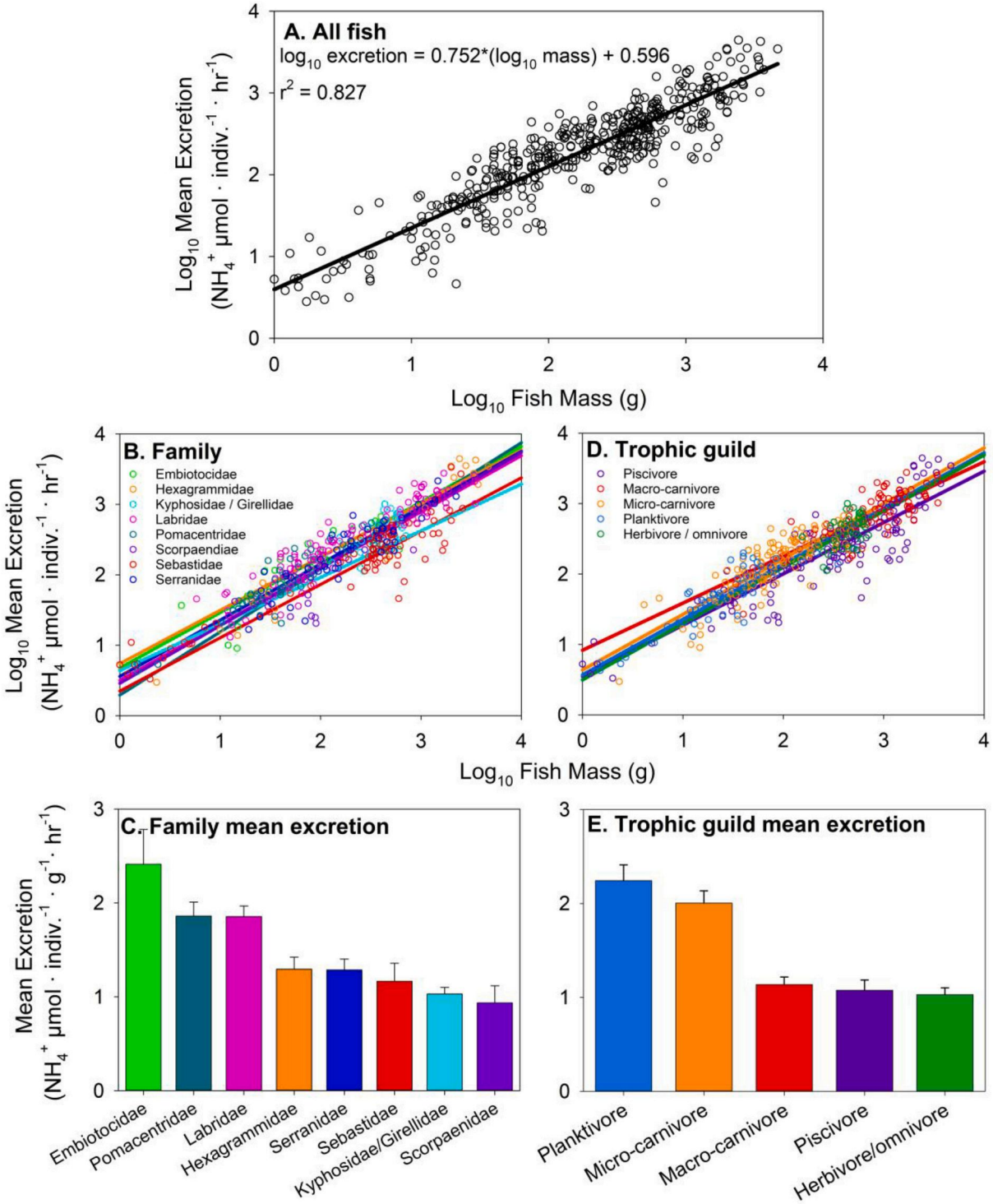

Fig. 5. (A) Linear regression depicting ammonium excretion rates of all fishes as a function of body mass. (B) Multiple linear regressions depict taxonomic variability in ammonium excretion by fish family as a function of body mass. Each point represents an individual fish, and each line represents a family. (C) Mass-specific mean excretion by family ±1 SE. (D) Multiple linear regressions depict variability in ammonium excretion by trophic group. (E) Mean mass-specific excretion rate by trophic group ±1 SE. Note: regression lines extended to the axes to aid visibility of elevation and slope.

bodied planktivores, and four times more ammonium compared to small-bodied micro-carnivores (Table 3).

4. Discussion

Ammonium excretion rates of kelp forest fishes were comparable with the empirically measured excretion rates of fishes in freshwater and tropical systems (Table 4). Kelp forest fish excretion rates (0.08–3.5 $\mu mol \cdot g^{-1} \cdot hr^{-1}$) were comparable to freshwater gizzard shad (0.4–7.2 $\mu mol \cdot g^{-1} \cdot hr^{-1}$; Mather et al., 1995), marine grunts (0.0016–2.5 $\mu mol \cdot g^{-1} \cdot hr^{-1}$; Meyer and Schultz, 1985), and tropical coral reef fishes (0.0001–19.5 $\mu mol \cdot g^{-1} \cdot hr^{-1}$; Allgeier et al., 2015). Notably, kelp forest fishes exhibited similar patterns as fishes in freshwater and tropical systems; excretion rates were positively correlated with fish wet mass,

Table 2
Mean excretion rates of kelp forest fishes by family.

			Wet Mass (g per individual)			Mass-specific excretion[a] ($\mu mol \cdot indiv^{-1} \cdot g^{-1} \cdot hr^{-1}$)	Per capita excretion[b] ($\mu mol\ NH_4^+ \cdot indiv^{-1} \cdot hr^{-1}$)
Family	**Species**	**n**	**Min**	**Mean**	**Max**	**Mean ± SE**	**Mean ± SE**
Hexagrammidae	Lingcod, Greenlings	26	2.33	1112	4670	1.29 ± 0.13	1136.0 ± 302.0
Myliobatidae	Bat Rays	11	1100	1888	2630	0.43 ± 0.02	837.7 ± 105.0
Cottidae	Cabezon	12	890	1383	2250	0.42 ± 0.07	581.2 ± 111.0
Labridae	Wrasses	112	5.81	518.1	3470	1.85 ± 0.11	553.8 ± 58.2
Kyphosidae	Opaleye, Halfmoon	43	130	440.5	705	1.03 ± 0.07	439.9 ± 33.3
Serranidae	Kelp bass	44	19.2	384.7	2240	1.29 ± 0.12	374.4 ± 62.4
Sebastidae	Rockfishes	81	0.3	549.4	2600	1.17 ± 0.19	317.5 ± 54.1
Malacanthidae	Ocean Whitefish	12	75	199.1	590	1.86 ± 0.42	297.5 ± 39.7
Embiotocidae	Surfperches	24	4.11	131.9	605	2.41 ± 0.37	213.2 ± 37.7
Pomacentridae	Damselfishes	64	0.4	133.6	820	1.86 ± 0.15	152.8 ± 18.6
Scorpaenidae	Scorpionfish	16	14	181.5	840	0.94 ± 0.18	131.6 ± 39.7
Gobiidae	Gobies	10	1.72	5.546	14.25	2.66 ± 0.88	9.3 ± 2.0
Labrisomidae	Island Kelpfish	5	4.9	9.685	21.18	0.77 ± 0.25	0.8 ± 0.2
	Total	**460**					

[a]Mass-specific excretion rates were calculated as total ammonium excretion divided by the wet weight (g) of an individual. [b]Per capita excretion rates were averaged over all sizes measured for that species. Species are arranged in order of decreasing per capita excretion rates. Sample size n = the number of incubated individuals. In the cases where the sample size of a specific species was not robust, individuals of similar taxonomic identity were grouped together.

Table 3
Mean excretion rates by trophic group.

			Wet Mass (g per individual)			Mass-specific excretion[a] ($\mu mol \cdot g^{-1} \cdot hr^{-1}$)	Per capita excretion[b] ($\mu mol \cdot hr^{-1}$)
Trophic group	**Species**	**n**	**Min**	**Max**	**Mean**	**Mean ± SE**	**Mean ± SE**
Macro-carnivore	CA Sheephead Kelp Greenling Pile Perch	62	33.8	3470	1066	1.26 ± 0.09	900.1 ± 81.55
Piscivore	Lingcod Kelp Bass CA Scorpionfish Cabezon Rockfishes	151	1100	2630	700.0	1.08 ± 0.11	513.7 ± 66.15
Herbivore/Omnivore	Opaleye Halfmoon	43	130	705	440.5	1.03 ± 0.07	440.0 ± 33.26
Micro-carnivore	Kelp Surfperch Black Surfperch Striped Surfperch Rainbow Surfperch Garibaldi Rock Wrasse Painted Greenling	117	2.33	820	136.4	2.00 ± 0.13	200.0 ± 13.96
Planktivore	Blacksmith Blue Rockfish Señorita	61	0.4	510	53.0	2.24 ± 0.17	74.1 ± 10.32
	Total	**434**					

[a]Mass-specific excretion rates were calculated as total ammonium excretion divided by the wet weight (g) of an individual. [b]Per capita excretion rates were averaged over all sizes measured for that species. Trophic groups are arranged in order of decreasing per capita excretion rates. Sample size n = the number of incubated individuals.

Table 4
A comparison of consumer-derived ammonium excretion rates in aquatic ecosystems.

Source	Year	System	Range	Mean	Notes
Shrestha et al.	This paper	Marine	0.08–3.5	0.7	30 kelp forest species
Peters et al.*	2019	Marine	0.01–3.41	0.47	14 kelp forest invertebrates
Bray et al.*	1986	Marine	2.1–3.3	N/A	*Chromis punctipinnis*
Bray et al.*	1988	Marine	0.5–7	0.9	Kelp forest; 5 fishes and 20 invertebrate species
Weisberg and Lotrich*	1982	Marine	N/A	12.5	*Fundulus heteroclitus*
McCarthy and Whitledge*	1972	Marine	N/A	9.58	*Engraulis ringens*
McCarthy and Whitledge*	1972	Marine	N/A	7.5	*Engraulis mordax*
McCarthy and Whitledge*	1972	Marine	N/A	3.75	*Trachurus symmetricus*
Durbin and Durbin	1981	Marine	N/A	0.54	*Brevoortia tyrannus*
Allgeier et al.	2015	Marine	0.0001–19.5	N/A	100 species of fishes and inverts in tropical systems
Meyer and Schultz	1985	Marine	0.0016–2.5	N/A	*Haemulid* spp.
Schaus et al.	1997	Freshwater	1.85–2.9	N/A	Gizzard Shad
Mather et al.	1995	Freshwater	0.4–7.2	N/A	Gizzard Shad
Mather et al.	1995	Freshwater	2.5–3.4	N/A	Bluegill (lab study)
Vanni et al.	2002	Freshwater	0.5–1.67	N/A	Gizzard shad and zebrafish

Range and mean values represent the mass-specific ammonium excretion rates ($\mu mol\ NH_4^+ \cdot g^{-1} \cdot hr^{-1}$). N/A = Value not reported. Asterisk (*) indicates studies that calculated excretion rates with the dry weight of the individual fish instead of wet weight.

but mass-specific excretion declined with increasing fish mass (Mather et al., 1995; Allgeier et al., 2015). Excretion rates of kelp forest fishes were also similar to the first values published for a subset of kelp forest fishes and invertebrates in southern California over thirty years ago (Table 4). Bray et al. (1986) reported that Blacksmith excreted ammonium at night (when not feeding) at a mean rate of 0.609–0.957 μmol·g wet weight^{-1}·hr^{-1} (using a conversion that dry mass is ~29% of wet mass), which declined further overnight due to fasting. We measured Blacksmith excretion of 1.63 ± 0.22 μmol·g wet wt^{-1}·hr^{-1} during the day, which was presumably higher due to active feeding. Bray et al. (1988) reported that the mass-specific excretion for a series of five fish species (California Sheephead, Kelp Bass, Blackeye Goby, Bluebanded Goby, and Island Kelpfish) ranged from 0.75 to 1.75 μmol·g wet wt^{-1}·hr^{-1}, which is comparable to the range we observed for a wider array of fish species and body sizes (0.1–3.5 μmol·g wet wt^{-1}·hr^{-1}). Peters et al. (2019) calculated ammonium excretion rates for 14 common benthic macroinvertebrates in southern California following similar methods used in this study and reported a range of mass-specific excretion rates for invertebrates (0.0025–0.85 μmol·g^{-1} wet weight·hr^{-1}). Although the mass-specific excretion ranges were similar to fishes, the per capita excretion ranges of invertebrates were dramatically different due to the increased body mass of fishes compared to most kelp forest invertebrates. Fishes excrete ammonium at faster rates than invertebrates (fishes: 5.9–2765 μmol·hr^{-1} versus invertebrates: 0.04–189 μmol·hr^{-1}), reflecting not only differences in mass and body composition, but their higher activity levels and metabolic rates (Vanni and McIntyre, 2016). Pairing invertebrate excretion measurements with those from fishes will enable further studies to investigate the impacts of complete consumer assemblages on nutrient cycling in kelp forests and to test the effects of anthropogenic stressors (e.g., fishing, climate change, habitat disruption) on the magnitude of nutrients excreted.

4.1. Fish contributions to kelp forest nitrogen cycling

The metabolic theory of ecology (MTE) predicts that body size and temperature determine differences in nutrient excretion rates among kelp forest fishes (Brown et al., 2004), while ecological stoichiometry (ES) predicts that diet and the dynamic balance of elemental composition between consumers and their prey determine the ratios of elements retained or excreted (Sterner and Elser, 2002). A recent meta-analysis of over 10,000 excretion rates from aquatic ectotherms (freshwater and marine invertebrates and vertebrates) attempted to unify these theories, finding that the best predictive model of nitrogen excretion included elements of both MTE and ES (Vanni and McIntyre, 2016). Body mass and temperature were the best predictors of excretion rates, with nitrogen excretion increasing with body mass and temperature. In addition, trophic guild and vertebrate classification (as a proxy for body nutrient content) were included in the best fit model, even though the MTE variables outperformed the ES variables as predictors (Vanni and McIntyre, 2016).

In our empirical measurements of ammonium excretion in kelp forest fishes, we found that individual excretion rates scaled to the 0.75 power of body mass, implying that increasingly larger fishes excreted nutrients at a comparatively slower rate for their size than smaller-bodied fishes. This result is in accordance with prior studies on animals in aquatic systems (Wen and Peters, 1994; Schaus et al., 1997), and is likely linked to metabolism, which also scales with the ¾ power of body size (Brown et al., 2004). The consumption and processing of prey is driven by metabolic demands, and thus is makes sense that the excretion of nutrient waste products would scale similarly to metabolism. A large survey of marine fish and invertebrates reported a ¾ power for both N and P excretion, after accounting for interspecific variation using mixed effect models (Allgeier et al., 2015), while a large meta-analysis of freshwater and marine invertebrate and vertebrate animals reported a lower scaling coefficient for nitrogen excretion (0.684) (Vanni and McIntyre, 2016). Under principles of MTE, it is predicted that organisms within a particular taxonomic group will excrete nutrients at similar rates due to relatively similar metabolic needs (Sterner and Elser, 2002; Vanni et al., 2002). Kelp forest fish excretion rates were similar among species within a family but varied significantly between fish families, similar to coral reef fishes (Allgeier et al., 2015), providing further support for the MTE in predicting excretion rates in kelp forest fishes. Nitrogen excretion rates are also influenced by temperature, with higher excretion rates in individuals from warmer environments (Vanni and McIntyre, 2016). Similarly, in our study ammonium excretion rates of fish families collected in southern California (Labridae, Pomacentridae) tended to be higher than those from central California (Hexagrammidae, Sebastidae), where water temperatures are ~5 °C cooler on average.

We tested the hypothesis that trophic group and other principles of ecological stoichiometry theory would also explain variation in ammonium excretion by fish consumers in kelp forests (Elser and Urabe, 1999). While we detected significant variation in nutrient excretion among trophic groups, we originally expected that the piscivorous predators, with higher nitrogen-rich diets, would excrete more ammonium than other trophic groups, which typically consume a lower-nitrogen diet. Instead, we observed higher mass-specific excretion rates for planktivores and micro-carnivores, compared to macro-carnivores, piscivores, and herbivore/omnivores. Vanni and McIntyre (2016) similarly reported that trophic guilds differed in nitrogen excretion rates, but opposite of their predictions, with invertivores and generalized carnivores having lower excretion rates than primary consumers, piscivores, and algae/detritivores. This result of high excretion rates by planktivores and micro-carnivores may be explained by the high intake rates of these consumers (Durbin and Durbin, 1981; Green and McFarland, 1994; Pinnegar et al., 2007) and the 100–300% faster digestibility of plankton and small invertebrates compared to fish prey, as has been observed in studies of Kelp Rockfish digestion (Van Dykhuizen, 1983). Relatively higher excretion in this guild may also be explained by the elevated activity levels and metabolic rates typical of planktivores, compared to the sit-and-wait and pursuit strategies typical of piscivores (Werner et al., 1986). Relative to their biomass, planktivores contribute disproportionately more to nitrogen supply on coral reefs than other trophic guilds (Allgeier et al., 2014), similar to what we observed in kelp forests. The relatively slow excretion rate by herbivore/omnivores may be explained by the slower digestion rate typical of these species in cool temperate ecosystems. The herbivores/omnivores included in this study are Kyphosids that are known in tropical systems to have a long gut and to use hindgut fermentation with microbial symbionts to break down relatively indigestible components (Pardesi et al., 2022). While it is not known precisely whether the California species use hindgut fermentation, previous studies have indicated that they exhibit a dietary shift from a primarily algal diet to a diet dominated by animal matter as temperatures decline (Behrens and Lafferty, 2012), highlighting the challenge of extracting sufficient nutrients from macroalgae to meet metabolic demands in cool temperate waters. Piscivores had low ammonium excretion rates in our study and the meta-analysis of Vanni and McIntyre (2016), potentially because these species tend to engulf their prey, digest those single prey items over prolonged periods of time, and because piscivores tend to be more likely to have empty guts than other trophic groups (Arrington et al., 2002), placing a higher percentage of those randomly field-captured fish in a fasted or basal excretion state. Piscivores on coral reefs also contribute disproportionately less to nitrogen supply than expected, based on their biomass (Allgeier et al., 2014).

Researchers testing the prevalence of the ecological stoichiometry theory in aquatic systems report conflicting results. Trophic level is a key determinant of excretion rates among fishes in lakes (Schindler and Eby, 1997), however it explains little variation in excretion among fishes in tropical streams (Vanni et al., 2002) or coral reefs (Allgeier et al., 2015). In the Caribbean, mesopredators (e.g., Haemulidae, Lutjanidae, Serranidae) excreted a lower ratio of nitrogen to phosphorous compared to herbivores, which was reflective of the more phosphorous-rich diet of

predators (Allgeier et al., 2013); however, subsequent studies revealed that trophic level is confounded by fish body mass and ultimately explained little of the overall variation in nutrient excretion (Allgeier et al., 2015). We classified fishes into broad trophic categories based upon adult feeding habits, intended to reflect an increasing rank order of dietary nitrogen content; however, many of the fishes in California kelp forests are opportunistic and generalist predators (Love, 2011). Further testing of nitrogen tissue content among kelp forest fish species, along with measurement of δ^{15} N values (as a proxy for trophic position), would be a worthy direction of future research to continue disentangling the effects of trophic guild on nutrient excretion in kelp forest assemblages.

For this study, we focused on the effects of body mass and taxonomic identity on nutrient excretion because these variables were the primary predictors of fish excretion in tropical systems (Allgeier et al., 2015); however, numerous additional variables may alter or interact with the effects of body size on fish-derived nutrient excretion rates in kelp forests. Excretion rates may vary by time of day and time since last feeding, as observed among Blacksmith (*Chromis punctipinis*), where ammonium excretion rates declined throughout the night after fish sought shelter and stopped feeding (Bray et al., 1986). Ontogenetic shifts in physiology and diet as an animal grows may also alter ammonium excretion rates and may explain some of the variability over the size range of a species. For example, freshwater American Gizzard Shad (*Dorosoma cepedianum*) excretion rates shift with diet and the subsequent variation in nitrogen to phosphorous ratios in the food, while excretion by Zebrafish (*Danio rerio*) is driven by ontogenetic changes in physiology (e.g., bone formation) as the fish ages (Pilati and Vanni, 2007). In addition, seasonal changes in prey availability may alter consumptive patterns (Van Dykhuizen, 1983), and excretion may be greater during periods when kelp canopies are large and food is readily available (summer-fall) compared to periods of storms and lower productivity (winter) when fishes may have empty stomachs (Roberts, 1979). Alternately, shifts from kelp forests to urchin barrens can dramatically change kelp forest food webs (Graham, 2004) and the types and quantity of prey consumed (Gabara et al., 2021), potentially altering rates of nutrient excretion. The choice of capture methods may impart handling stress on the fish, which could alter excretion rates. However, for the three capture methods we used, we did not detect any differences in excretion, at least for Kelp Bass and California Sheephead. For one species, Lingcod, we included excretion estimates from 4 captive individuals at our lab (out of the 10 sampled). Contrary to expectations that field capture would elevate excretion due to handling stress (Whiles et al., 2009), mass-specific excretion rates were higher (1.49 ± 0.17 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ lab vs. 0.78 ± 0.1 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ field), not lower in these captive individuals. Caution should be used before interpreting these results further, given the very low sample size and multitude of potential confounding factors. Additional testing of variables that influence nutrient excretion rates will help explain variability within and among kelp forest fish species. For example, differences in water temperature between central California (range of 10–16 °C) and southern California (range of 14–20 °C) during our study period, could contribute to differences in excretion, as ammonium excretion increases with temperature, similar to metabolism (Wood, 2001; Vanni and McIntyre, 2016). However, this is confounded by the inherent species- and family-level differences in excretion that correspond with changes in fish assemblage structure between central and southern California (Horn and Allen, 1978; Miller, 2023). None of the species we sampled were caught in both regions and thus we could not test the effects of temperature on excretion rates in the field, while controlling for taxonomic differences. Therefore, controlled laboratory studies are required to further disentangle the effects of temperature on excretion rates in this system and how excretion may change in response to marine heatwaves, El Niño events, or other climatic change. Fortunately, because the fish faunas are generally distinct between central and southern California (Horn and Allen, 1978; Miller, 2023), using our equations to estimate ammonium excretion should be accurate for the typical temperature and environmental conditions of the regions where each species occurs.

4.2. Considerations for field incubation experiments

The calculation of accurate excretion rates is essential for linking consumers to biogeochemical processes; however, determining the appropriate duration for incubation experiments with fishes is not well established in the literature. No single incubation period is optimal for all study organisms and the proper duration for an incubation is widely contested in the literature. For example, an observed decrease in consumer excretion rates during longer experiments (>2 h) are attributed to elevated initial excretion rates caused by stress from perceived predation (Kiesecker et al., 1999) or handling (Whiles et al., 2009) and exercise or exhaustive activity (Randall and Wright, 1987). However, others argue that a decrease in excretion rate is due to incipient fasting effects that lead to an underestimation of natural excretion rates (Lehman, 1980; Devine and Vanni, 2002; Glaholt and Vanni, 2005; Whiles et al., 2009). As the ideal methods to quantify ammonium excretion by kelp forest fishes have not been previously established, we recommend that fish incubate for at least 30 min during experiments, and that researchers conduct experimental time trials to determine the appropriate incubation period for the species of interest. These recommendations are in agreement with procedures and recommendations established in Whiles et al. (2009) that extensively tested the effects of fasting and handling effects on excretion rates over multiple incubation periods in freshwater Brook Trout (*Salvelinus fontinalis*) and a recent meta-analysis of empirical nutrient excretion rates in 10,000 ectotherms (median duration of 1.3 h across all taxa measured). A 30-min incubation period is also widely used in excretion experiments for tropical marine fishes (Allgeier et al., 2014) and temperate invertebrates in kelp forests (Peters et al., 2019). For kelp forest fishes, empirical excretion measurements of 30-min duration are appropriate; our results indicated that excretion did not change over the course of an hour (measured in 10-min time bins) and did not depend on the length of the incubation duration since the start of the experiment. However, we did observe increased variability in excretion rate estimates within the first 10–20 min of starting an incubation, thus, we recommend incubations are conducted for a minimum of 20 min. These results are in accordance with tropical fish nutrient excretion rates that asymptote within 30 min and remained relatively constant thereafter (Allgeier et al., 2014), balancing the need to reduce effects of handling stress with those of fasting.

4.3. Conclusion

This study provides detailed estimates of the rates of ammonium excretion for the dominant fish species inhabiting shallow rocky reefs and kelp forests in California. We found that ammonium excretion rates depended on fish body mass, family identity, and trophic group affinity, suggesting that maintaining large-bodied fishes, as well as taxonomic diversity, could have important effects on nearshore nitrogen cycling in kelp forests. The development of family-specific predictive excretion rate equations provides a framework for modeling the contribution of fish-derived nutrients by the complete fish community in kelp forests on an areal scale, as well as the assessment of the spatiotemporal factors that drive nutrient excretion in California kelp forests (Shrestha, 2020). In addition, calculations of consumer derived nutrient excretion at the community level will provide an opportunity to evaluate the role of fishes in the broader nitrogen cycle in kelp forests, comparing the magnitude of nutrients excreted by consumers to physical delivery processes such as upwelling and internal waves (Brzezinski et al., 2013). Recent work suggests that urea may be an additional important source of nitrogen in kelp forests (Smith et al., 2018), thus, additional testing of other nutrients excreted by fishes (e.g., urea, creatine, and phosphorous), will give a greater context to the role of consumer-derived nutrients in kelp forest ecosystems. This study is an important step forward

in understanding the contribution of the full fish community to nutrient cycling in kelp forests, however, much more work remains to understand the overall role of consumers in this system.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by J. Shrestha and S. Hamilton. The first draft of the manuscript was written by J. Shrestha and S. Hamilton and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Code availability

Not applicable.

Ethics approval

All fish collections were permitted by the California Department of Fish and Wildlife (Permit: Hamilton #SC-6477). The San Jose State University Institutional Animal Care and Use Committee approved the protocols for the capture and handling of fish (protocol #1034).

Author statement

The enclosed work has not been published or accepted for publication, and is not under consideration for publication in another journal or book. Submission of this manuscript for publication has been approved by all relevant authors and institutions and all persons entitled to authorship have been so named.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

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