California Fish and Game 94(1):18-32 2008

SPAWNING TIME, FECUNDITY, HABITAT UTILIZATION, AND PARASITES OF A NORTHERN CALIFORNIA POPULATION OF TIDEWATER GOBY, *EUCYCLOGOBIUS NEWBERRYI*

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ABSTRACT

***Eucyclogobius newberryi*, the tidewater goby, is a Federally Endangered species endemic to coastal lagoons and estuaries in California. Little is known about the biology of this species in northern California; thus we conducted a 14- month study (November 2004-December 2005) to assess spawning time, fecundity, habitat utilization, and parasites of a northern California tidewater goby population in Big Lagoon, Humboldt County, CA. The presence of individuals less than 20 mm indicated that spawning occurred every month except March and April. Gravid females were observed in March and May-July 2005; fecundity ranged from 190 to 531 eggs. Goby habitat utilization was evaluated on a seasonal basis using a stratified random sampling technique. High goby densities were associated with vegetation in silt and sand substrates. Minimum population estimates of tidewater gobies in the southern end of Big Lagoon were highest in winter 2005 (N=2714) and lowest in fall 2005 (N=163). Parasitological analysis revealed the presence of a newly described microsporidian species infecting muscle tissue of the tidewater goby. A total of 105 of 1513 fish were visibly infected. Visibly infected gobies ranged in total length between 19 and 56 mm and were observed in every month except February 2005.**

INTRODUCTION

Eucyclogobius newberryi, the tidewater goby (Teleostei:Gobiidae), is a species endemic to California coastal lagoons and estuaries. The tidewater goby range is from Tillas Slough (mouth of Smith River, Del Norte County) south to Agua Hedionda

Lagoon (San Diego County) (Moyle 2000). Historic records suggest that the tidewater goby once occurred in as many as 124 locations (Fish and Wildlife Service, 1994[1]). There are currently 96 known populations, of which 42 are thought to be nearly extirpated (Fish and Wildlife Service, 1994[1]). In 1994, the tidewater goby was listed as an Endangered species under the U.S. Endangered Species Act (Fish and Wildlife Service, 1994[1]).

Previous life history studies of tidewater gobies have been focused in the southern and central part of its range (Swift et al. 1989; Swenson 1999). No studies have explicitly examined the biology of northern California populations. Such a study is warranted, as northern California populations are genetically and morphologically differentiated from southern populations. Molecular phylogenetic analyses have revealed that tidewater goby populations in northern California (Del Norte, Humboldt, and Mendocino counties) are genetically distinct from all other tidewater goby populations (Dawson et al. 2001). Morphological analysis of the lateral line system of the supraorbital region are generally consistent with these findings, revealing that northernmost populations (Del Norte, Humboldt, and Mendocino counties) of tidewater gobies are morphologically distinct from other populations (San Diego County) (Ahnelt et al. 2004). The objectives of this study were to assess spawning time, fecundity, habitat utilization, and parasites infecting the tidewater goby.

STUDY AREA

Big Lagoon (41°9'86" N, 124°7'85" W) is located 9 km north of Trinidad, California. Big Lagoon is approximately 6 km long and 3 km wide and is separated from the Pacific Ocean by a 600-700 m wide sand bar. Maple Creek and an unnamed tributary provide freshwater inputs to the lagoon. The Highway 101 causeway runs through the eastern portion of the lagoon near the confluence with Maple Creek. Along the east side of the lagoon, substrate consists of boulders. Along the west, lagoon substrate is primarily sand and gravel. Substrate in the south, near Big Lagoon Rancheria, includes clay, gravel, sand, and silt. There is a small unnamed tributary with silt substrate near the Humboldt County Big Lagoon Park boat launch ramp. Vegetation in the lagoon includes bulrushes, *Scirpus* spp., submerged terrestrial vegetation, *Carex* spp., and aquatic vegetation including widgeongrass, *Ruppia maritima*.

In the fall and winter, heavy rains typically cause the lagoon to flood and water levels reach three to four m above mean sea level (Kraus et al. 2002). When high lagoon levels combine with heavy oceanic storm swells the sand bar separating the lagoon from the ocean opens (breaches), connecting the lagoon with the ocean. Excess flood water drains from the lagoon over a period of 24 hours, often with high initial rates of discharge (Kraus et al. 2002). Following a breach, the lagoon becomes tidally influenced and salinities often increase (Kraus et al. 2002). Two breaches in Big Lagoon occurred in the northwestern corner during the study period: 1 January 2005, and 16 November 2005.

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[1] U.S. Fish and Wildlife Service. 1994. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for the Tidewater Goby. Federal Register 59: 5494.

MATERIALS AND METHODS

Spawning Time

Sampling was focused at the southern end of Big Lagoon near the Humboldt County Big Lagoon Park boat launch, near the mouth of the small unnamed tributary. Tidewater gobies were sampled with a 3.3 m x 1.3 m x 0.158 cm mesh seine with leads spaced every 10 cm in depths between 0.5- 1.5 m. Approximately 100 gobies were sampled on a monthly basis for 14 months. Total length (TL) was recorded to the nearest mm. During each sampling period, surface temperature and salinity were also measured (depth < 1 m); temperature was recorded to the nearest °C and salinity to the nearest ‰ using a refractometer.

A length-frequency histogram of goby TL was constructed for each monthly sampling period. For monthly samples containing only one apparent size class, mean TL was estimated from raw length data. MIX Software (http://www.math.mcmaster.ca/peter/mix/mix31.html, version 3.1, Brinbook, Ontario) was used to analyze histograms with overlapping size class distributions. Initial parameter values for MIX analysis were set at two components, with equal proportions (0.5).

Fecundity

Fecundity of tidewater goby in Big Lagoon was determined by counting eggs of nine females, collected between March and July. These individuals were euthanized with MS-222 and their ovaries were removed and preserved in 95% ethanol. Eggs were counted under a dissecting microscope. A sub-sample of 10 eggs each from 6 individuals was measured. Egg diameter was determined using an ocular micrometer (0.001 mm scale) with a light microscope under 10x magnification.

Habitat Utilization

The technique of stratified random sampling (Thompson 2002) was employed to estimate tidewater goby density within microhabitats of southern Big Lagoon. A total of nine strata were defined by substrate and the presence or absence of vegetation (silt vegetated, sand vegetated, sand unvegetated, sand-gravel vegetated, sand-gravel unvegetated, sand-clay vegetated, sand-clay unvegetated, boulder vegetated, and boulder unvegetated). Sampling units consisted of 5-m sections of shoreline within constructed habitat strata. Gobies were collected at each site by pulling a seine (3.3 m x 1.3 m x 0.158 cm mesh, with leads spaced every 10 cm) a distance of 5 m. It was assumed that this method of collection provided a minimum count of tidewater gobies present in the area sampled. Habitat variables of salinity, temperature, substrate type, vegetation type, % vegetative cover, % organic matter (determined by presence of water surface leaf litter and woody debris), and an average of three depths were measured at each sampling unit. Within each habitat strata h, there are N_h sampling units consisting of 5-m segments of shoreline. At each seasonal sampling period, n_h sampling units were selected from habitat stratum *h*, with larger sample sizes selected from strata with higher tidewater goby density (vegetative cover, sand and silt substrate).

The perimeter of southern Big Lagoon was scouted using a small motorized skiff to identify strata transition points. Transition points between strata were discerned visually and mapped using a Global Positioning System unit (Fig. 1). Transect lengths were calculated using ArcGIS (version 9.1, Redlands, California). Strata transition points were consistent through time. However, not all strata were sampled each season due to changing water levels (some strata were dry whereas others were sometimes too deep to be sampled). Sampling effort was concentrated in strata that contained the highest tidewater goby density (vegetative cover, sand, and silt substrate).

Habitat sampling was conducted seasonally in winter (19 February), spring (13 May), summer (28 August), and fall (5 November) of 2005. A minimum population estimate $\hat{Y}_T$ of tidewater gobies along the shoreline of southern Big Lagoon was made by first calculating mean number of gobies per seine haul in habitat strata stratum *i*, ($\overline{D_i}$) defined as:

$$(1) \qquad \overline{D_i} = \frac{N_i}{n_i}$$

where N_i = total number of gobies caught in stratum *i* and n_i = number of seine hauls in stratum *i*. A minimum population estimate ($\hat{Y}_i$) for each individual stratum was calculated as:

$$(2) \qquad \hat{Y}_i = \frac{L_i}{5} \times \overline{D_i}$$

where L_i = distance (in meters) of habitat stratum *i*, (determined by using ArcGIS, v. 9.1, Redlands, California)(Table 1) and $\frac{L_i}{5}$ is the number of sampling units in habitat stratum *i*. A minimum population estimate across all nine strata ($\hat{Y}_T$) was calculated by:

$$(3) \qquad \hat{Y}_T = \sum_{i=1}^{9} Y_i$$

$\hat{Y}_T$ was calculated separately for each season.

RESULTS/DISCUSSION

Spawning Time

Spawning time in Big Lagoon was assessed using monthly length-frequency histograms. Tidewater goby eggs take between 9-10 days to hatch, and settle out of the plankton a maximum of 14 days later at 16-18 mm TL (Swift et al. 1989). Thus, it can be inferred that individuals 20 mm or less are new recruits to the population spawned approximately 23-24 days earlier. Individuals 20 mm TL or less were noted in all months except April, May, and October - December 2005 (Figs. 3, 5 and Table 1). These data

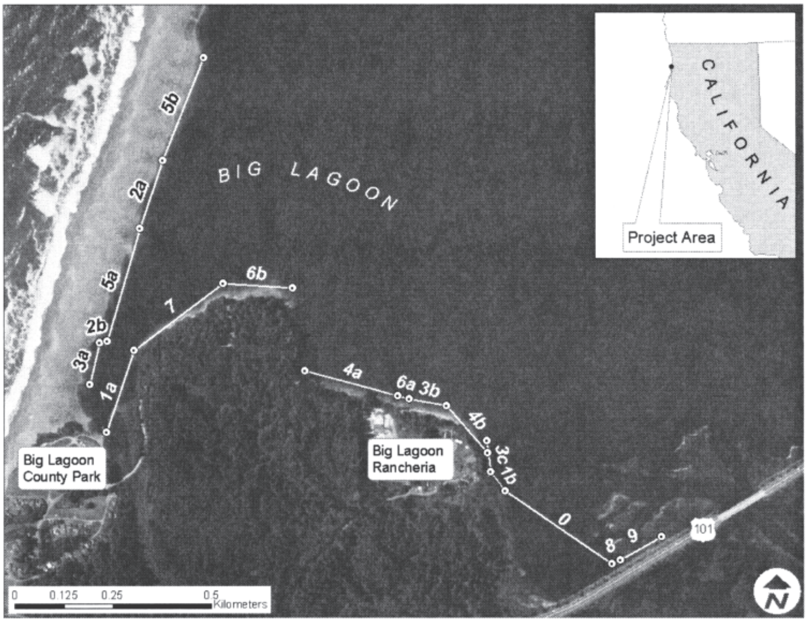
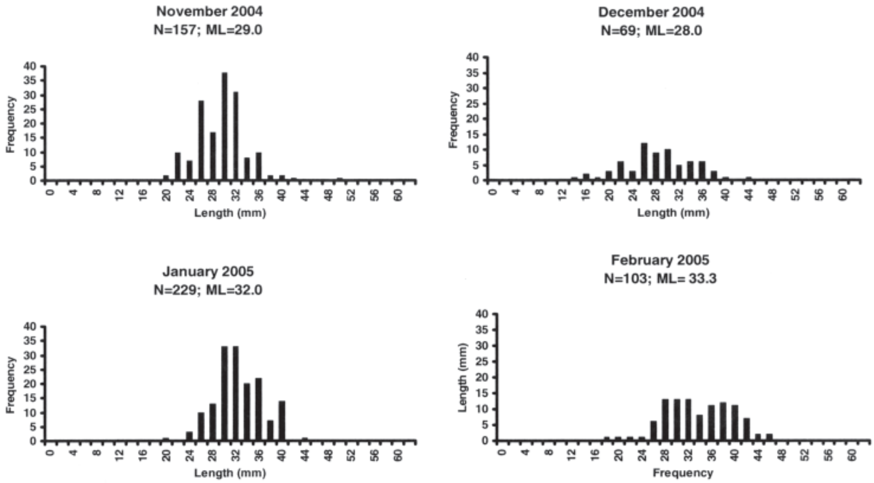
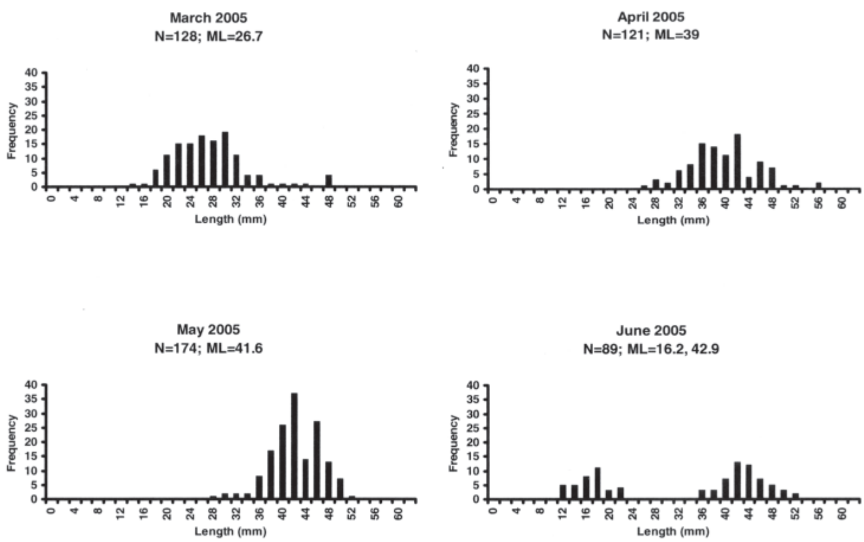


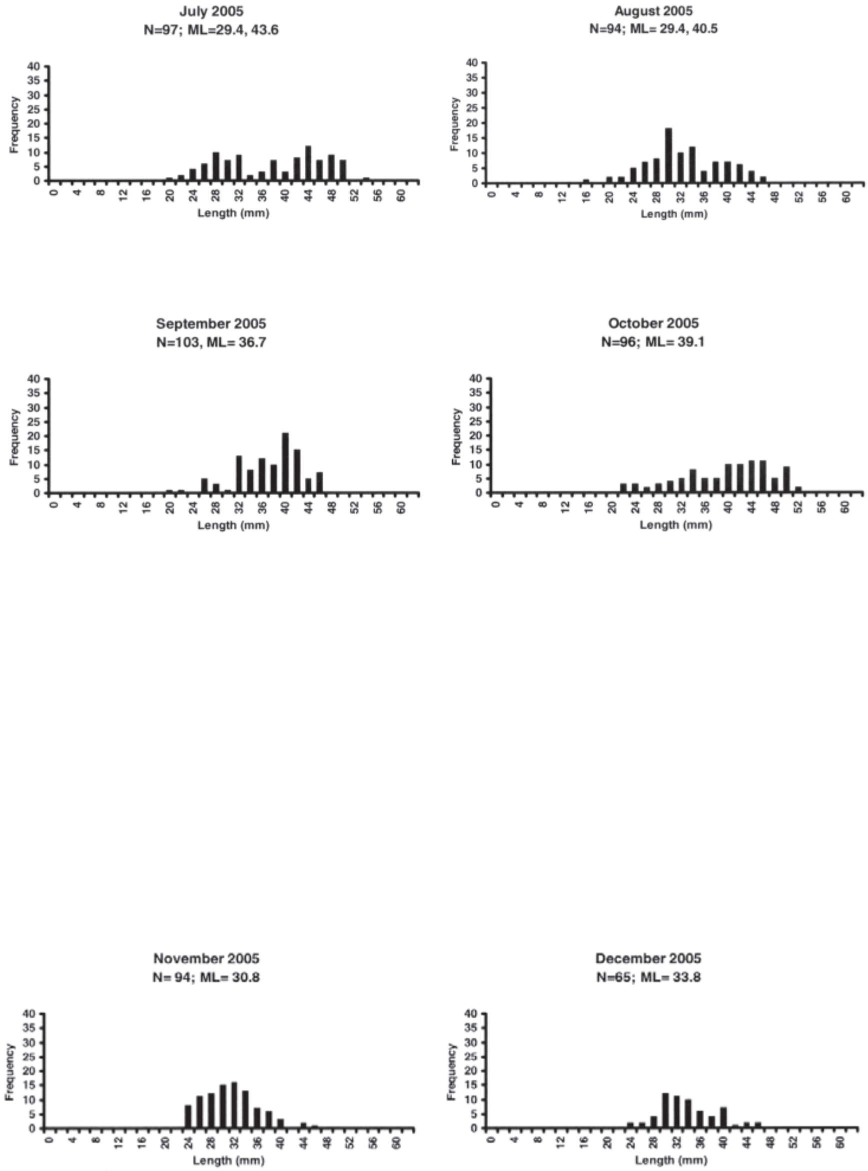
Figure 1: Location of habitat strata sampled on a seasonal basis in southern Big Lagoon. 3a (110 m) = sand veg, 2b (20 m) = sand unveg, 5a (299 m) = sand gravel unveg, 2a (182 m) = sand unveg, 5b (282 m) = sand gravel unveg, 1a (220 m) = silt vegetated, 7 (284 m) = sand clay unveg, 6b (178 m) = sand clay veg, 4a (245 m) = sand gravel veg, 6a (30 m) = sand clay veg, 3b (97 m) =sand veg, 4b (159 m) = sand gravel veg, 3c (80 m) = sand veg, 1b (61 m) = silt veg, 0 (303 m) = bulrushes, 8 (23 m) = boulder veg, and 9 (121 m)= boulder unveg.

Table 1: Habitat strata transect distance for seasonal stratified sampling.

Strata	Transect	Distance (m)
SILT-VEG	1A	220
	1B	61
SAND-UNVEG	2A	182
	2B	20
SAND-VEG	3A	110
	3B	97
	3C	80
SAND-GRAVEL VEG	4A	245
	4B	159
SAND-GRAVEL UNVEG	5A	299
	5B	282
SAND-CLAY VEG	6A	30
	6B	178
SAND-CLAY UNVEG	7	284
BOULDER-VEG	8	23
BOULDER-UNVEG	9	121


November 2004
N=157; ML=29.0
Frequency
Length (mm)
December 2004
N=69; ML=28.0
Frequency
Length (mm)
January 2005
N=229; ML=32.0
Frequency
Length (mm)
February 2005
N=103; ML= 33.3
Length (mm)
Frequency


March 2005
N=128; ML=26.7
Frequency
Length (mm)
April 2005
N=121; ML=39
Frequency
Length (mm)
May 2005
N=174; ML=41.6
Frequency
Length (mm)
June 2005
N=89; ML=16.2, 42.9
Frequency
Length (mm)



Figures 2-5: Monthly length-frequency histograms of tidewater goby. N = sample size; ML = mean total length (mm).

suggest that some spawning can occur during almost any month with the exception of March and April. Further, these data indicate that spawning time varies from year to year. For example, in 2004 spawning occurred in October-December; however, in 2005 no spawning was evident during these same months.

Length-frequency data showed two distinct size classes from June - August 2005 (Figs. 3, 4) consistent with a spawning gap in April 2005. These size classes are termed cohort 1 (2004-2005 year class) and cohort 2 (2005-2006 year class). Cohort 1 increased in TL from November 2004 through July 2005, where it reached a maximum mean TL of 43.6 mm (Fig. 6). In August 2005, the decrease in mean TL to 40.5 mm (Fig. 4, 6) is hypothesized to be due to larger individuals dying after spawning. Cohort 2 appeared in June 2005 (Figs. 3, 6). Cohorts 1 and 2 overlapped in September and October 2005 (Figs. 4, 6) and were not separable by MIX analysis. By November 2005 it appeared as if most of cohort 1 had died and only cohort 2 remained, evident as a uni-modal size class (Fig. 5).

Length-frequency histograms with two distinct size classes suggesting a spawning gap have been observed in other parts of the tidewater goby range. Two distinct age classes were observed during the month of June in Aliso Creek Lagoon, Orange County (Swift et al. 1989). This pattern was also evident in the spring and summer in San Gregorio and Pescadero lagoons in San Mateo County (Swenson 1999), and in May

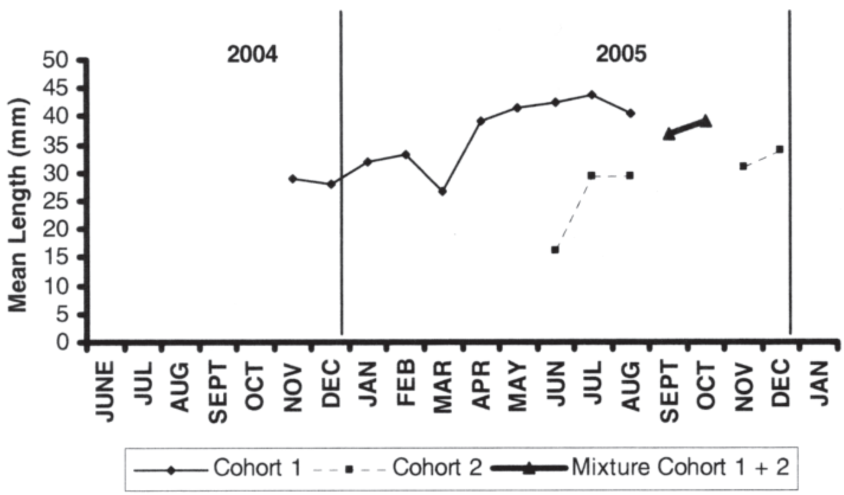


Figure 6: Mean monthly total length (mm) of tidewater goby November 2004- December 2005. Cohort 1= 2004-2005 age class, Cohort 2= 2005-2006 age class, Cohort 1+2= mixture of 2004-2005 and 2005-2006 age classes.

in Pico Lagoon, San Luis Obispo County (Worcester 1992[2]). Thus, it appears that it is possible to find tidewater gobies spawning during the majority (more than 9 months) of the year.

Fecundity

Gravid females were observed in March (N=1), May (N=2), June (N=2), and July (N=4). Egg numbers varied from 190 to 531 per gravid female. Egg diameter ranged from 0.37 to 0.79 mm, with an average diameter of 0.61 mm (STD=0.122). Eggs in the same ovary varied in diameter in by as much as 0.2 mm, suggesting that fractional spawning occurs. Females have been observed to spawn multiple times (Swenson 1999). Observed egg numbers and diameters are consistent with previous studies, where fecundity has been shown to range from 179- 594 eggs (Swift et al. 1989) and from 362-1010 eggs (Swenson 1999), and egg diameter has ranged from 0.5 to 1 mm (Swift et al. 1989).

Habitat Utilization

Tidewater goby were collected from a broad range of salinities and temperatures in Big Lagoon. Salinity ranged from 0 to 13 ‰ (Fig. 7) during the 14-month study period. A minimum salinity of 0 ‰ was recorded in June, November, and December 2005. A maximum salinity of 13 ‰ was recorded in April. Breaching events (1 January and 18 November, 2005) did not appear to affect salinity at the study site (Fig. 7). Temperature ranged from 10° to 25° C (Fig. 8). Recorded temperature slowly increased over each monthly sampling from November 2004 to a maximum of 25° C in July 2005. Recorded temperature steadily declined from July 2005 to December 2005. Minimum temperatures of 10° C occurred in November 2004 and December 2005. Like salinity, temperature was not greatly affected by the two breaching events (Fig. 8). These findings are comparable with other tidewater goby populations in Pescadero Creek and Lagoon, San Mateo County and Aliso Creek Lagoon, Orange County, wherein temperatures of goby habitats ranged from 8-25° C and salinities ranged from 0-41 ‰ (Swift et al. 1989; Swenson 1995[3]).

Tidewater goby were also collected from a broad range of habitats in Big Lagoon. Six of the nine habitat strata sampled contained at least one tidewater goby during one of the seasonal sampling periods (Table 2, Figs. 9, 10). However, preferred tidewater goby habitat in Big Lagoon, as indicated by strata with the largest number of goby per seine haul, were the silt-vegetated and sand-vegetated habitats (Table 2). The silt-

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[2] Worcester, K.R. 1992. Habitat Utiliziation in a central California coastal lagoon by the tidewater goby (*Eucyclogobius newberryi*). M.S. thesis. California Polytechnic State University, San Luis Obispo, CA, USA.

[3] Swenson, R.O. 1995. The reproductive behavior and ecology of the tidewater goby *Eucyclogobius newberryi* (Pisces: Gobiidae). Ph.D. thesis. University of California, Berkeley, CA, USA.

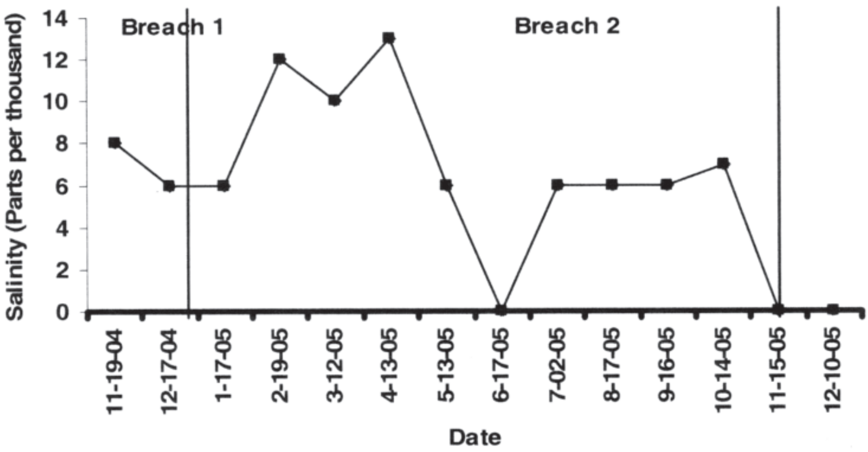

Figure 7: Monthly recorded salinity (‰) from November 2004-December 2005.

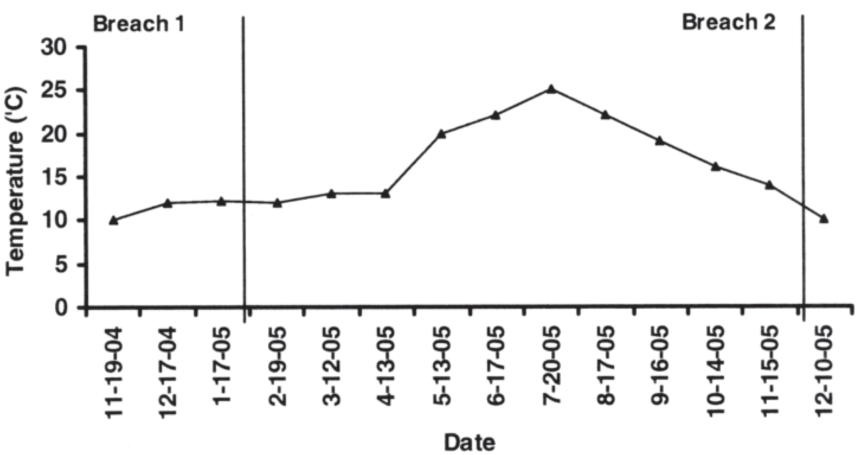

Figure 8: Monthly recorded temperature (°C) from November 2004-December 2005.

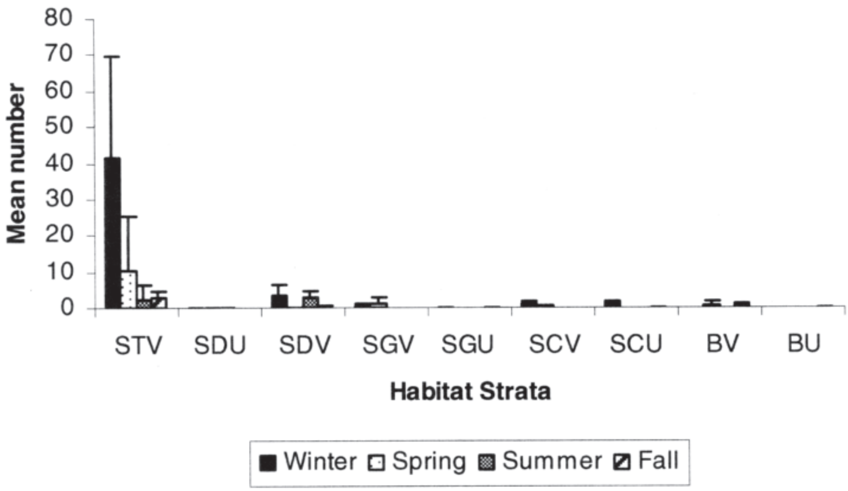

Figure 9: Mean number of goby per 5-m seine haul ($\overline{D_i}$) by strata and season. STV= silt vegetated; SDV= sand vegetated; SDU= sand unvegetated; SGV=sand gravel vegetated; SGU=sand-gravel unvegetated; SCV=sand-clay vegetated; SCU= sand-clay unvegetated, BV= boulder vegetated, and BU=boulder unvegetated.

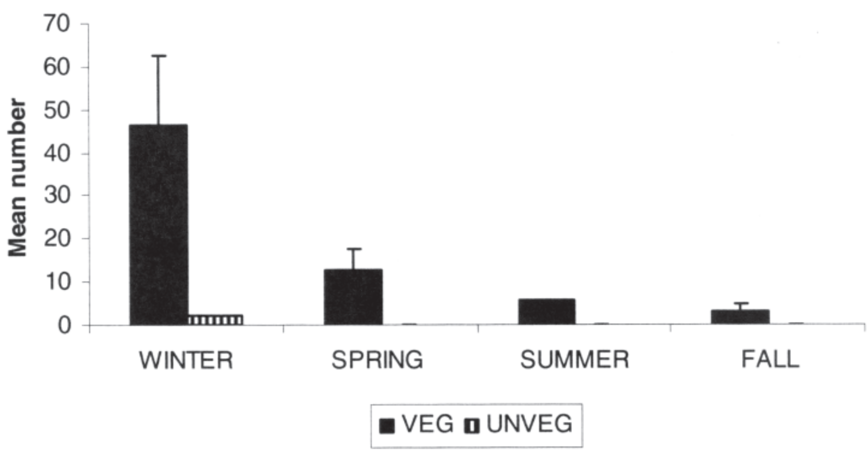

Figure 10: Mean number of goby per 5-m seine haul ($\overline{D_i}$) in vegetated and unvegetated strata by season.

Table 2: Mean goby number per 5-m seine haul ($\overline{D_i}$) by strata and season. Standard deviation in parentheses. (-) indicates strata was not sampled.

Strata	Winter	Spring	Summer	Fall
STV	41.5 (28.05)	10.42 (14.89)	2.5 (3.78)	2.67 (1.77)
SDU	0 (0)	0 (0)	0 (0)	-
SDV	3.25 (3.3)	0 (0)	3 (1.73)	0.2 (0.44)
SGV	0.5 (0.7)	1.25 (1.5)	-	-
SGU	0	-	-	0 (0)
SCV	1 (1)	0.33 (0)	-	-
SCU	2 (0)	0	-	0 (0)
BV	-	0.75 (0.957)	-	0.4 (0.89)
BU	-		-	0 (0)

vegetated habitat stratum had mean goby numbers of 41.5 gobies per seine haul in the winter, 10.4 gobies per seine haul in the spring, 2.5 gobies per seine haul in the summer, and 2.7 gobies per seine haul in the fall. The silt-vegetated habitat was the only strata which gobies were found in all four seasons. Overall, gobies occurred in strata with vegetation, and did not occur in strata without vegetation (with the exception of the sand-clay unvegetated strata with a mean number of two gobies per seine haul in the winter seasonal sampling)(Table 2).

The Big Lagoon tidewater goby population was largest in the winter and gradually declined throughout the year. A decrease in mean goby numbers was observed from winter 2005 to fall 2005 in all habitat strata (Fig. 9). Further, minimum population estimates of gobies in southern Big Lagoon gradually decreased throughout the year from 2,714 in the winter, 704 in the spring, 313 in the summer, and 163 in the fall (Table 3). Large fluctuations in goby population numbers are not unprecedented, but, other studies show an opposite pattern with the highest goby numbers in summer and fall, and lowest numbers during the winter (Irwin and Soltz 1984[4], Swift et al. 1989, Worcester 1992[2], Swenson 1995[3], Fong 1997[5]). Tidewater goby's unique reproductive strategy of early sexual maturation, spawning multiple times, and an almost complete annual turnover in individuals is likely responsible for the high inter-annual variation in population sizes (Swenson 1999). However, movement of the tidewater goby to different habitats during different times of the year may also be a possible explanation (Swenson 1999).

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[4] Irwin, J.F. and Soltz, D.L. 1984. The natural history of the tidewater goby, *Eucyclogobius newberryi*, in the San Antonio and Shuman Creek Systems, Santa Barbara County, CA. U.S. Fish and Wildlife Report #11310-0215-2.

[5] Fong, D. 1997. 1996 tidewater goby (*Eucyclogobius newberryi*) sampling in Rodeo Lagoon, Golden Gate National Recreation Area, Marin County. National Park Service Report.

Table 3: Months with new tidewater goby recruits (< 20 mm TL) and inferred spawning time.

Month	< 20 mm TL	Inferred Spawning
October 2004		x
November 2004		x
December 2004	x	x
January 2005	x	x
February 2005	x	x
March 2005	x	
April 2005		
May 2005		x
June 2005	x	x
July 2005	x	x
August 2005	x	x
September 2005	x	
October 2005		
November 2005		
December 2005		

Parasites

A newly described species of microsporidium, *Kabatana newberryi*, (McGourty et al. 2007) infected the Big Lagoon tidewater goby study population. Infection was indicated by white globular structures just beneath the epidermal surface; 7 % (105/1,513 individuals) of the sample population were visibly infected. This represents a minimum infected rate as individuals are likely infected but the microsporidium are too small to be visually discerned. Visibly infected individuals were found every month of the sampling period except February 2005 (Fig. 11). The range in TL of visibly infected individuals was 19-56 mm and the average TL of visibly infected individuals was 37.3 mm (Fig. 12). This suggests that *Kabatana newberryi*, at a relatively advanced state of infection, occurs in settled-out larvae as well as adults at nearly all times of the year in Big Lagoon.

A similar visible infection of white globular structures just beneath the epidermal surface has been observed in populations throughout Del Norte, Humboldt, and Mendocino counties and Rodeo Lagoon, Marin County (G. Goldsmith, unpublished data; D. Fong, National Park Service, personal communcation). Histopathological effects of these microsporidia on the tidewater goby are currently unknown. Further investigation is needed to determine how this microsporidium may impact the long-term persistence of the endangered tidewater goby.

ACKNOWLEDGMENTS

I thank Dr. D. Hankin for his assistance in planning my sampling design, clarifying my data results, and greatly improving the quality of this manuscript. I would also like

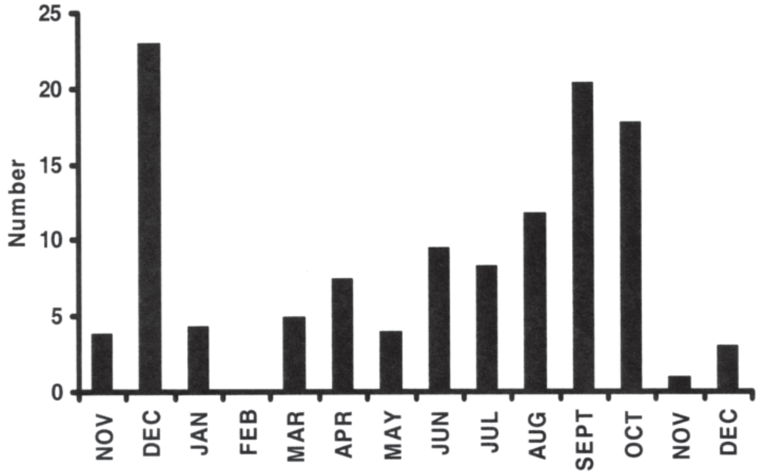

Figure 11: The number of tidewater gobies visibly infected with *Kabatana newberryi* on a monthly basis from November 2004-December 2005.

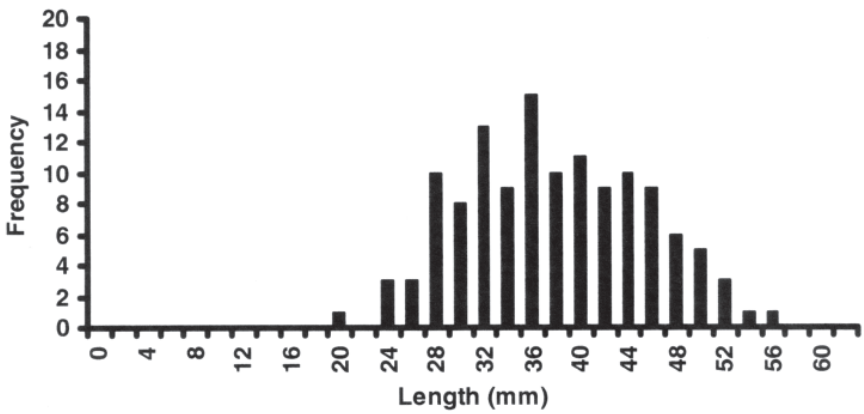

Figure 12: Length-frequency histogram of tidewater gobies visibly infected with *Kabatana newberryi* from November 2004-December 2005.

Table 4: Minimum population estimates of tidewater goby by season and strata. Mean number of gobies per seine haul in parentheses.

Strata	Winter	Spring	Summer	Fall
STV	2332.3 (41.5)	586 (10.4)	140.5 (2.5)	150 (2.67)
SDU	-	-	-	-
SDV	186.55 (3.25)	-	172.2. (3)	11.48 (0.2)
SGV	40.4 (0.5)	101 (1.25)	-	-
SGU	-	-	-	-
SCV	41.6 (1)	13.87 (0.33)	-	-
SCU	113.6 (2)	-	-	-
BV	-	3.45 (0.75)	-	1.84 (0.4)
BU	-	-	-	-
Min Pop Est	2714.45	704.4	312.7	163.4

to thank co-author G. Goldsmith for assisting me in obtaining the necessary permits in a timely fashion, and for his assistance in the field. I thank the many individuals who assisted me with my field work including C. Hannon, J. Gough, D. Alvarez, D. Goodman, T. Fuller, C. Meredith, M. Scott, M. Torres, and E. Fox.

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Received: 28 September 2006
Accepted: 12 March 2007