

# Effects of sewage effluent discharge on the abundance, condition and mortality of hulafish, *Trachinops taeniatus* (Plesiopidae)

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## Abstract

The effects of sewage effluent on the abundance, condition (length and reproduction) and mortality of hulafish *Trachinops taeniatus* were investigated at multiple outfall and control locations on the central coast of New South Wales, Australia. Underwater visual surveys found consistently fewer *T. taeniatus* at locations where sewage was discharged compared to control locations. The condition of *T. taeniatus* was investigated by comparing mean length and reproductive indices of fish from two outfall and two control locations. Fish from the largest outfall location were significantly smaller in comparison with fish from control locations. Gravid female fish from outfall locations had similar gonadosomatic indices but a significantly greater number of eggs and smaller size of eggs in comparison with fish from control locations. Mortality of *T. taeniatus* was investigated during 2-week, in situ, caging experiments at multiple locations and times and 80% of fish survived, although mortalities of up to 73% per cage were recorded at one sewage outfall. *T. taeniatus* may be a suitable environmental indicator species of sewage pollution. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Fish; Sewage; Abundance; Condition; Length; Reproduction; Mortality; NSW, Australia

## 1. Introduction

Environmental pollution and its effects on the state of our aquatic resources continues to be a problem of growing, world-wide concern (Earle, 1995; Baird, 1996; Koop and Hutchings, 1996). The most common cause of point source pollution to the world's waterways is the disposal of sewage (Dubinsky and Stambler, 1996). The early detection or forecasting of human impacts on the distribution and abundance of organisms will enable effective management of coastal environments (Jones and Kaly, 1996) and ensure the survival of aquatic ecosystems and associated activities, such as fisheries and coastal tourism. A principal challenge posed in assessments of environmental impacts is to isolate the effect of interest from spatial and temporal variability (Underwood and Peterson, 1988; Schmitt and Osenberg, 1996).

A range of ecological responses in fish have been attributed to sewage pollution including: increased

mortality, increased or decreased abundance or diversity, and changes to size, reproduction, contaminant levels, parasites, infections or behaviour (Tsai, 1975; Love et al., 1987; Grigg, 1994, 1995; Siddall et al., 1994; Lemly, 1996). Many authors have investigated the impacts of sewage on fish abundance and/or condition (Adams, 1990; Gray et al., 1992; Otway, 1995; George et al., 1995; Svanberg, 1996), but few have conducted field experiments. The field experiments have generally placed freshwater fish, such as salmonids and cyprinids, in cages adjacent to sewage outfalls and these fish generally suffered high mortalities (Tsai, 1975; Mitz and Giesy, 1985; Kakuta and Murachi, 1997).

The idea that a single taxon may be used to indicate broader impacts on the community is now widely accepted (Stephens et al., 1988; Jones and Kaly, 1996). Two different types of species are generally used as indicators of pollution (Keough and Quinn, 1991). Positive indicators are opportunistic species that may occur in large numbers in polluted areas, either because environmental conditions become favourable for these species and/or detrimental to other species or because superior competitors are removed. Examples of such species include green algae, particularly *Ulva* spp.

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(Fairweather, 1990; Hardy et al., 1993; Bokn et al., 1996), the polychaete worm *Capitella capitata* (Schmitt and Osenberg, 1996) and carp *Cyprinus carpio* (Tsai, 1975). Negative indicators are species that are common in relatively unpolluted areas and decline in abundance in response to pollution; examples are the seagrass *Posidonia* spp. (Chisholm et al., 1997) and perhaps butterflyfishes (Hourigan et al., 1988). The hulafish *Trachinops taeniatus* is the only species of fish that has been recognised as a potential bioindicator of sewage pollution in coastal waters of Australia (Smith et al., 1999).

The eastern hulafish *T. taeniatus*, Gunther 1861 (Plesiopidae), is a common rocky reef fish endemic to Australian waters from Noosa (Queensland) to Cape Conran (Victoria). It is a schooling, planktivorous species which attains a maximum total length of around 10 cm (Hutchins and Swainston, 1986). *T. taeniatus* has no direct value as a commercial or recreational food or game fish, although it is sought by collectors of aquarium fish.

To investigate the effects of sewage discharge on *T. taeniatus*, we used a multidisciplinary approach that involved estimates of population abundance, measures of condition of individual fish, and a field experiment to measure mortality.

## 2. Materials and methods

### 2.1. Abundance of *T. taeniatus*

*T. taeniatus* were surveyed at three regions off the central coast of New South Wales (NSW) (Fig. 1). All regions were surveyed using the same methodology, personnel and in similar subtidal habitats. The time of surveys (between July 1992 and February 1998) and size and treatment of the outfalls differed (TEL, 1994; Smith et al., 1999), and we therefore present results from the three regions as separate studies:

1. Hunter region. Three locations were surveyed with controls at Tomaree Head and Point Stephens, and the impact location was a small outfall at Boulder Bay (Fig. 1a), discharging 4.3 Ml/day of secondary treated sewage (MHL, 1997).
2. Sydney region. Four locations were surveyed, with controls at North Head and Gordons Bay and the impact locations were outfalls at Rosa Gully discharging 5.1 Ml/day (raw sewage), and Potter Point discharging 46 Ml/day (primary sewage) (Fig. 1b).
3. Illawarra region. Three locations were surveyed, with controls at Flinders Island and Atchinsons Rock and the impact location was an outfall at Port Kembla, 16.8 Ml/day (primary sewage) (Fig. 1c).

Fish were surveyed by scuba divers in the boulder or barrens habitat (Underwood et al., 1991) at a depth range of 12–15 m. Within each location, fish were surveyed along four 60-m long transects. Each transect was laid parallel to the shoreline, and located within 100 m of the outfall. Fish were sampled during daylight hours during periods of low swell (less than 1 m) and when water visibility exceeded 5 m. *T. taeniatus* were counted 1 m either side of the transect line (total area 120 m<sup>2</sup>) (Lincoln Smith, 1988, 1989). Schools of *T. taeniatus* appeared to have a restricted home range and were not visibly attracted or repelled by a scuba diver (personal observation).

Data were tested for homogeneity of variance by Cochran's test, transformed to  $\log(x+1)$  if variances were heterogeneous, and analysed using analysis of variance (ANOVA). For the Hunter region a three-way nested asymmetrical ANOVA (beyond Before After Control Impact (BACI) design; Underwood, 1994) was used to compare fish among periods (before, immediately after commissioning, and 1 year after commissioning of the sewage outfall), times (four times within each period) and locations (one outfall and two controls). For the Sydney region a two-way ANOVA was used to compare fish among times (two) and locations (two outfalls and two controls) and, similarly, a two-way ANOVA was used to compare fish among times (four) and locations (one outfall and two controls) for the Illawarra region. Significance of means were tested using Ryan's test.

### 2.2. Condition of *T. taeniatus*

Fish were only collected in the Sydney region at two control locations: North Head and Gordons Bay and two outfalls: Rosa Gully and Potter Point (Fig. 1a). Fish were not collected in the Hunter and Illawarra regions. Fish were collected by a scuba diver with two small (30-cm diameter) hand-held dip nets on five occasions between February 1996 and February 1998. At least 100 fish were captured from each site at each time ( $n = 6657$ ). Total lengths of all fish were measured in the laboratory on the same day they were collected using callipers (0.01 mm). For all fish collected in 1996 and December 1997 ( $n = 3130$ ), we recorded the number of gravid females ( $n = 83$ ) and measured their gonad weights, and by using a binocular microscope we counted the numbers of eggs and measured the diameters of a subsample of 20 eggs from each female. Gonadosomatic index (GSI) was calculated as gonad weight/total weight  $\times 100$ . A small proportion of gravid female fish did not have distinct eggs. The sex of juveniles was not determined and male gonads were not weighed as the largest were less than 0.01 g.

Data for total length, weight, GSI, egg number, egg diameter were tested for homogeneity of variance by Cochran's test, transformed if necessary, and analysed

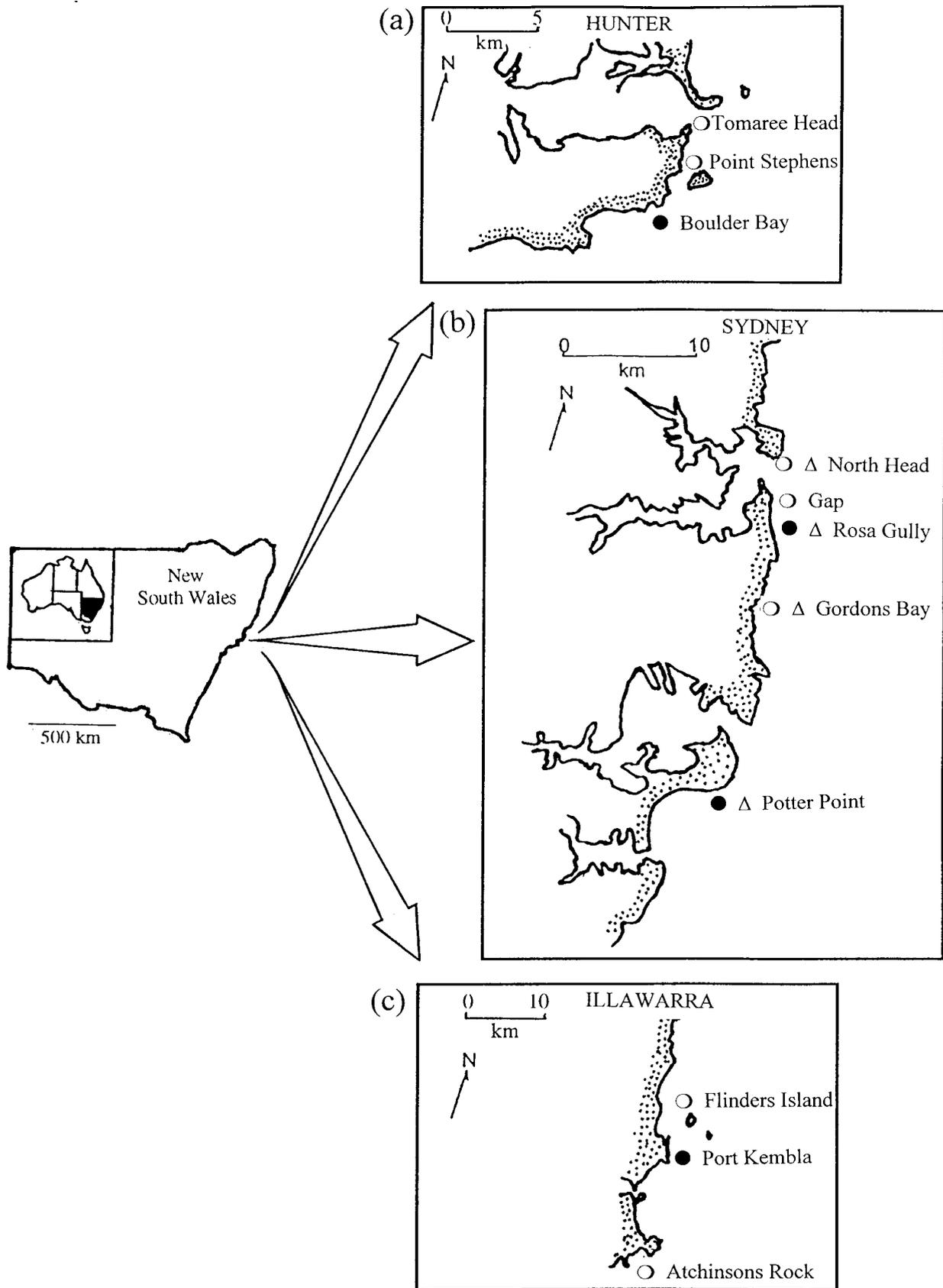


Fig. 1. Study locations for surveys of *Trachinops taeniatus* in the (a) Hunter, (b) Sydney and (c) Illawarra regions. The locations in the Sydney region marked with  $\Delta$  were also used for the condition and mortality studies. Sewage outfalls are indicated by black symbols.

using ANOVA. Significance of means were tested using Ryan's test.

### 2.3. Mortality of *T. taeniatus*

Mortality was investigated by placing fish in cages at control and outfall locations in the Sydney region (Fig. 1a). Cages were constructed according to a lantern cage design. A pilot study determined that this cage design appeared robust in coastal swells of less than 2 m. Cages were supported by a 15 cm foam float and anchored by a 25 kg concrete block. The cages were constructed of 1-m deep cylinders of 6 mm raschel netting which were supported by two stainless steel rings of 40 cm diameter. The netting was fastened at the top and bottom by a drawstring.

Two cages were deployed at each of two control and two sewage outfall locations (Fig. 1a) for approximately 2 weeks during both March 1996 and March 1997 (total of 16 samples). Cages containing 15 fish captured from the same location (Gap; Fig. 1b) were deployed in the rocky reef habitat in water depths of approximately 12–15 m. Cages at the sewage outfall locations were deployed within 50–100 m of the outlet pipe and within the sewage plume. All cages were inspected on the day following deployment and all fish were alive. Cages were retrieved after 2 weeks and fish were counted.

Data on survivorship were tested for homogeneity of variance by Cochran's test, transformed if necessary, and analysed using ANOVA. Significance of means were tested using Ryan's test.

## 3. Results

### 3.1. Abundance of *T. taeniatus*

A total of 1539 *T. taeniatus* was counted in the Hunter region. ANOVA indicated that there was a significant location $\times$ period effect (Table 1). As the sewage outfall at Boulder Bay was commissioned on 15 November 1993, the abundance of *T. taeniatus* clearly declined by at least 50% after commissioning of the sewage outfall at the impacted location (Fig. 2a).

A total of 1220 *T. taeniatus* was counted in the Sydney region, and the total abundances of *T. taeniatus* at the control locations were significantly greater by 300–600% than at the outfall location at Potter Point (Table 2, Fig. 2b). The abundance of *T. taeniatus* at the outfall location at Rosa Gully was similar to the control location at Gordons Bay but significantly less than the control location at North Head (Fig. 2b).

A total of 3547 *T. taeniatus* was counted in the Illawarra region. Greater abundances of fish were recorded from the control locations by 300–500% compared to the outfall locations (Table 3, Fig. 2c).

Table 1

Summary of beyond Beyond After Control Impact (BACI) analysis of variance of the abundance of *Trachinops taeniatus* from the Hunter region at one outfall and two control locations, three periods (before, immediately after commissioning, 1 year after commissioning—four times in each period) and with four replicates

Source of variation	df	MS	F	p <sup>a</sup>
Period (P)	2	2.78	11.31	****
Time(Period) (T(P))	9	0.54	0.96	ns
Location (L)	2	5.76	23.46	***
Outfall vs control locations (O vs C)	1	2.26	0.24	ns
Between control locations (C)	1	9.26	38.92	*
P $\times$ L	4	0.63	2.55	*
P $\times$ O vs C	2	0.74	1.44	ns
P $\times$ between C	2	0.51	2.16	ns
T(P) $\times$ L	18	0.51	2.08	**
T(P) $\times$ O vs C	9	0.57	1.23	ns
T(P) $\times$ between C	9	0.46	1.87	ns
Residual	108	0.25		

<sup>a</sup> ns,  $p > 0.05$ ; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

### 3.2. Condition of *T. taeniatus*

From a total of 6657 *T. taeniatus*, we measured at least 100 fish from each of four locations and five times (Fig. 3). The total lengths of all fish ranged from 13 to 98 mm, with a mean length of 29.6 mm ( $\pm 0.15$  SE). One way ANOVA indicated that significantly larger fish were captured at the control location at Gordons Bay and significantly smaller fish were captured at the sewage outfall at Potter Point (Table 4, Fig. 4).

Relatively small sample sizes were obtained to investigate the impacts of sewage on the reproductive condition of fish. Only 25 ripe female *T. taeniatus* were collected from a total of 1494 fish (1.7%) in February–March 1996, and 58 ripe females were collected from a total of 1636 fish (3.5%) in December 1997. We therefore pooled the two temporal samples into outfalls and controls locations prior to analysis. There was no difference in the length, weight or GSI of gravid female *T. taeniatus* from outfall and control locations (Table 4). We detected significant differences in the number of eggs and the egg diameter (Table 4). The number of eggs per female was greater at outfall locations by approximately 15% and the mean diameter of eggs was approximately 10% less at the outfall locations (Fig. 5).

### 3.3. Mortality of *T. taeniatus*

Of the 16 cages deployed, 14 were retrieved and two were lost. The overall mortality of *T. taeniatus* was 19%; however, this ranged from 0 to 73% mortality (15 to four fish) for individual cages (Table 5). The greatest mortality was recorded from a cage at the Potter Point outfall. ANOVAs were conducted separately for the mortality in 1996 and 1997, and the combined years and

these showed no significant effects, although  $p = 0.07$  was near significant for an outfall effect, and the power of the analysis was low (0.36 for year and 0.19 for the interactions).

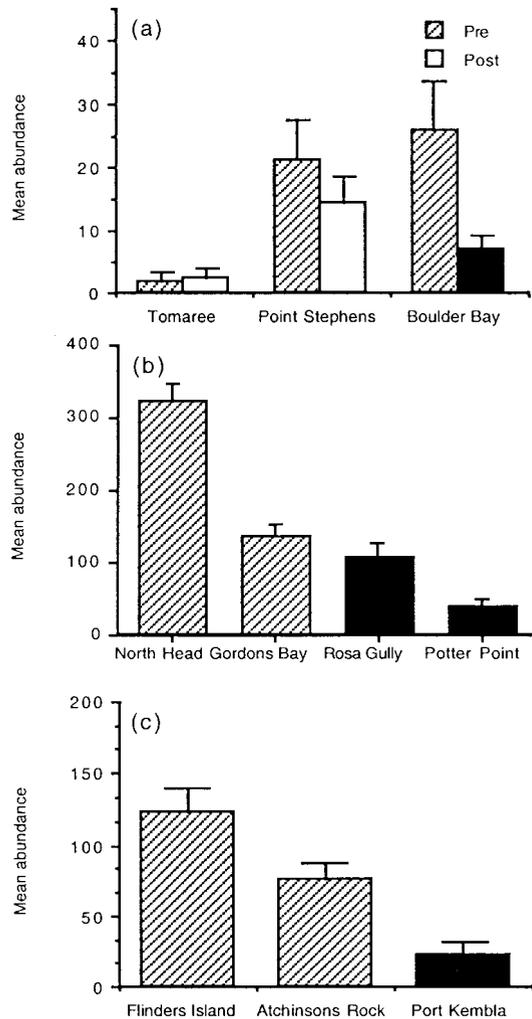


Fig. 2. Mean abundance ( $\pm$  SE) of *Trachinops taeniatus* (per 120 m<sup>2</sup>) at outfall and control sites in the (a) Hunter (striped bars indicate pre-commissioning, white bars indicate post-commissioning at control locations and black bars indicate post-commissioning of the sewage outfall), (b) Sydney, and (c) Illawarra regions. (Sewage outfalls are indicated by black bars and striped bars include control locations).

Table 2

Summary of analysis of variance of the abundance of *Trachinops taeniatus* from the Sydney region at two outfall and two control locations, two times and with four replicates

Source of variation	df	MS	F	$p^a$
Time	1	0.01	0.04	ns
Location	3	1.57	7.10	***
Time $\times$ location	3	0.18	0.81	ns
Residual	24	0.22		

<sup>a</sup> ns,  $p > 0.05$ ; \*\*\*  $p < 0.001$ .

## 4. Discussion

### 4.1. Abundance of fish impacted by sewage

We found decreased abundances of *T. taeniatus* at the outfall sites compared to the control sites for the three regions. The relative abundance of *T. taeniatus* was largest at control locations and smallest at sewage locations and therefore abundance may be regarded as a negative indicator of sewage pollution. Other negative indicators of sewage pollution include the seagrass *Posidonia* spp. (Chisholm et al., 1997) and perhaps butterflyfishes (Hourigan et al., 1998). The increased or decreased abundances of fish adjacent to sewage outfalls may be site or species specific (Pilanowski, 1992; Grigg, 1994; Otway, 1995; Smith et al., 1999). Other studies have reported that sewage impacts on fish populations may result in a replacement of valuable species (gadoids, flatfish and lobsters in marine areas, and salmonids and sturgeon in freshwater/estuarine habitats), by lower-value small pelagic fish and perciforms, respectively (Caddy, 1995). Decreased abundances of Australian snapper *Pagrus auratus* and increased abundances of gurnard *Lepidotrigla mulhali* were consistent patterns attributed to deepwater ocean outfalls, while the abundance of mosaic leatherjacket *Eubalichthys mosaicus* increased at one outfall and decreased at another off Sydney (Otway, 1995).

The scale of impact of sewage on the abundance of *T. taeniatus* varied from approximately 50 to 600%, with a smaller impact at the small outfall in the Hunter region and larger impacts at the larger outfalls in the Illawarra and Sydney regions. There was large variability in the abundance of *T. taeniatus* among control locations, particularly for the Hunter region. In this region we used a beyond BACI experimental design (Underwood, 1994) which allowed the detection of significant differences before and after the impact.

Table 3

Summary of analysis of variance of the abundance of *Trachinops taeniatus* from the Illawarra region at one outfall and two control locations, four times and with four replicates

Source of variation	df	MS	F	$p^a$
Time	3	2853	1.55	ns
Location	2	2687	1.46	ns
Outfall vs control locations (O vs C)	1	5148	22.9	ns
Between control locations (C)	1	225	0.70	ns
Time $\times$ location	6	1838	2.26	ns
T $\times$ O vs C	3	3356	10.40	*
T $\times$ between C	3	321	0.39	ns
Residual	37	813		

<sup>a</sup> ns,  $p > 0.05$ ; \*  $p < 0.05$ .

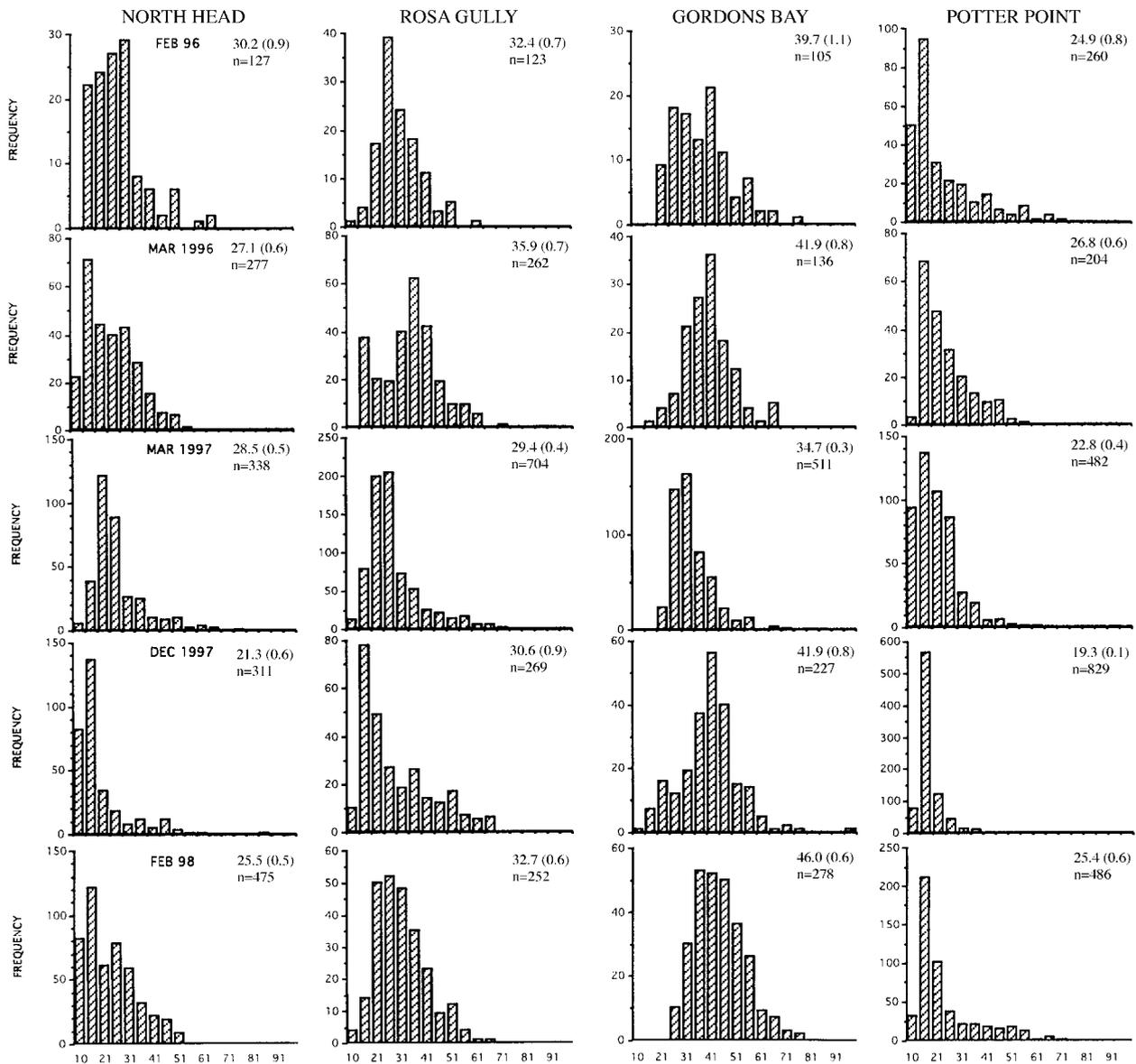


Fig. 3. Length frequency distribution (total length, mm) of *Trachinops taeniatus* from four locations in the Sydney region and five times (5-mm increments). Note the scale of the y-axis varies ( $n = 6657$ ).

#### 4.2. Condition of fish impacted by sewage

We found consistent patterns of smaller fish from the larger coastal outfall at Potter Point and significantly larger fish at a control location at Gordons Bay. Possible explanations for the smaller mean size of fish observed at the sewage outfall at Potter Point may be increased mortality, slower growth, dispersal of larger fish away from the sewage, increase in the predation on larger *T. taeniatus*, or preferential recruitment. These explanations appear to be supported by the length frequency distributions of *T. taeniatus* (Fig. 3), which show a large abundance of recently recruited *T. taeniatus* (less than 20 mm) at Potter Point at all times, and very low abundances of large fish.

Although only a small proportion of *T. taeniatus* were gravid females, the fish from the outfall locations had a greater mean egg number and smaller egg diameter. Other studies have reported a range of impacts of sewage on fish reproductive condition (Donaldson, 1990; Waring et al., 1996; Lye et al., 1997). For example, a substantial proportion of the male flounders *Platichthys flesus* exposed to sewage effluent had malformed testes (Lye et al., 1997). Sand gobies *Pomatoschistus minutus* were exposed to sewage sludge and no significant effect was found on GSI; however, there was a major reduction in the number of eggs and larvae produced in the sludge-exposed population which reflected a failure of some females to spawn (Waring et al., 1996).

Table 4

Summary of two-way analysis of variance (ANOVA) of condition (length) and one-way ANOVA of condition (reproduction) indices for *Trachinops taeniatus* at two outfall and two control locations in the Sydney region

Source of variation	df	MS	F	p <sup>a</sup>
<i>Length</i>				
Location	3	65537	675	***
Time	4	5453	56	***
Location × time	12	2678	27	***
Residual	6637	97		
<i>Reproduction</i>				
Length	1	135	3.55	ns
Residual	78	38		
Weight	1	0.168	0.912	ns
Residual	78	0.184		
Gonadosomatic index	1	48.7	1.48	ns
Residual	78	33.0		
Number of eggs	1	177197	4.05	*
Residual	59	43764		
Diameter of eggs	1	0.819	38.6	***
Residual	578	0.021		

<sup>a</sup> ns,  $p > 0.05$ ; \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ .

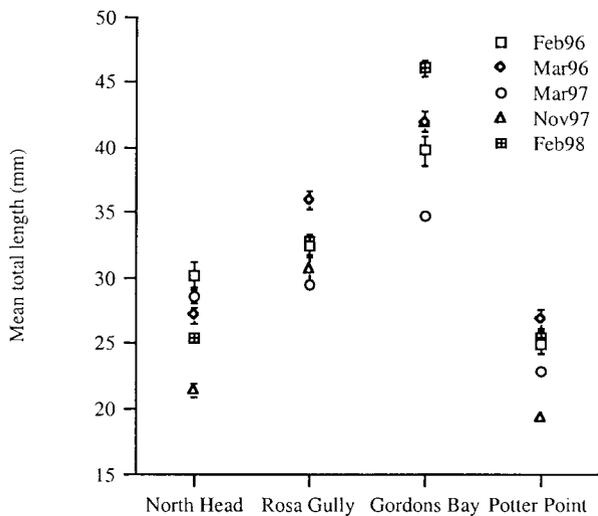


Fig. 4. Average total length ( $\pm$ SE) of *Trachinops taeniatus* from four locations in the Sydney region and five times ( $n = 6657$ ).

#### 4.3. Mortality of fish impacted by sewage

Most *T. taeniatus* survived the experimental manipulation of caging; however, a large proportion (73%) died in one of the cages at the sewage outfall at Potter Point in 1996. Unfortunately, the loss of several cages reduced the power of the statistical tests, and no significant impact could be attributed to sewage pollution. The experimental design could have been improved if we had additional replicate cages (i.e. four cages per location instead of two) to increase the power of the analysis.

Table 5

Number of *Trachinops taeniatus* (out of a total of 15 per cage) which survived experimental caging at control and outfall locations in the Sydney region

		1996		1997	
		1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	2 <sup>a</sup>
Control	North Head	11	15	13	11
	Gordons Bay	9	15	14	x <sup>b</sup>
Outfall	Rosa Gully	13	12	15	14
	Potter Point	4	x <sup>b</sup>	11	13

<sup>a</sup> Cage number.

<sup>b</sup> Missing cage.

The relatively short experimental periods of 2 weeks was selected because previous researchers had demonstrated rapid mortality of fish exposed to sewage. For example, mortality of juvenile chinook salmon *Oncorhynchus tshawytscha* occurred at all sites within 4.4 km of a 1530 Ml/day sewage treatment plant outfall which discharged to the Fraser River, British Columbia (Birtwell et al., 1983). Mortality was rapid, and fish placed 2.2 km from the outfall died within 9 min as a result of low dissolved oxygen (Birtwell et al., 1983). Experimental caging studies also demonstrated that acute mortality of channel catfish *Ictalurus punctatus* occurred at sites 300 and 500 m downstream from a 76 Ml/day sewage treatment plant outfall on the Flint River, Michigan (Mitz and Giesy, 1985). However, three species of rock fish were placed in cages in a sewage effluent zone in San Francisco Bay and no significant mortality could be demonstrated on fish after 96 h of exposure to 1% effluent (Tsai, 1975).

#### 4.4. Future research and management

*T. taeniatus* were considered to be a good organism to study because they are a resident rocky reef species which are abundant, easy to capture and their small size and planktivorous feeding were important for achieving the practical limitations of maintaining fish in cages.

Three avenues of research deserve attention in the light of these findings. The scale of impact on abundance of *T. taeniatus* varied according to the different regions and locations with declines from approximately 50 to 600% in comparison to control locations. Surveys of the abundance of this species have been undertaken at four ocean outfalls, and there are over 40 outfalls in NSW waters. The cumulative impact of sewage discharge in NSW waters on the abundance of this species should be investigated.

Secondly, differences in condition (mean length and reproductive indices) of *T. taeniatus* at outfalls and control locations and the generality of these patterns compared to other, more distant and pristine locations

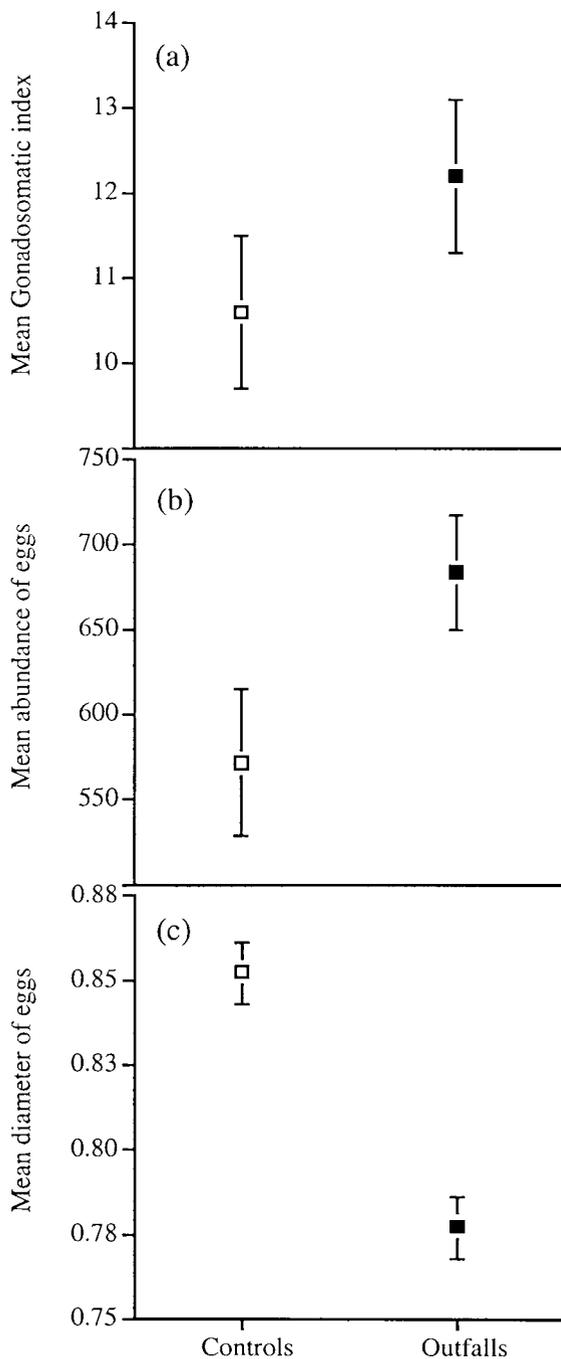


Fig. 5. Reproductive indices ( $\pm$ SE) of female *Trachinops taeniatus* from control and outfall locations in the Sydney region. (a) Mean Gonadosomatic index ( $n = 79$ ), (b) mean abundance of eggs ( $n = 60$ ), and (c) mean diameter of eggs ( $n = 580$ ). The samples from outfall and control locations have been pooled.

should be investigated. The samples for the condition study were only collected in the Sydney region and the selection of control sites may be partially confounded by the impact of diffuse sources of pollution, such as stormwater. Diffuse sources of pollution were not measured but were likely to be considerable for the site at North Head at the entrance to Sydney Harbour, and this

may explain why the length of fish from this control location were significantly smaller than the control location at Gordons Bay. *T. taeniatus* had more eggs and smaller eggs at outfall locations and laboratory experiments could be initiated to determine spawning success.

Thirdly, the use of bioindicators or sentinel animals in cages to monitor pollution has given rise to the establishment of national and international programmes (Phillips and Segar, 1986; Avery et al., 1996; Jones and Kaly, 1996). To date these programs have focused on using oysters and mussels in Australian waters (Scanes et al., 1995; Avery et al., 1996), although fish have been used in overseas studies (Tsai, 1975; Mitz and Giesy, 1985; Stephens et al., 1988). Although the *T. taeniatus* experiments did not detect a significant impact on mortality, we suggest that this may be partially due to the low power of the experimental design and the loss of replicate cages. It is logical to monitor a species that is very abundant in the coastal waters of NSW, in preference to the existing monitoring programs which transplant an estuarine species, the Sydney Rock oyster *Saccostrea commercialis* into a coastal environment.

In conclusion, this research detected different scales of impact of sewage on *T. taeniatus* with approximately 50–600% declines in abundance, increases and decreases of 10–50% in condition indices (length, reproduction) although no detectable impact on mortality. While other studies have focused on the effects of sewage on fish abundance (Gray et al., 1992; Otway et al., 1996), condition (Waring et al., 1996; Kakuta and Murachi, 1997; Lye et al., 1997) or mortality (Lemly, 1996), this is one of the few studies to have used a multidisciplinary approach to investigate the impacts of sewage pollution (but see Liu and Morton, 1998). Similar multidisciplinary studies should be undertaken on other species of fish and biota that are exposed to sewage pollution. The identification of the effects of sewage on *T. taeniatus* increases the choice of possible bioindicators in Australian waters.

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