Mass Mortality of Captive Southern Bluefin Tuna (*Thunnus maccoyii*) in April/May 1996 in Boston Bay, South Australia: A Complex Diagnostic Problem

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In April 1996 approximately 75% (1,700 t) of the captive, southern bluefin tuna *Thunnus maccoyii* in Boston Bay, South Australia died. Most deaths occurred during the two days, 15 and 16 April. There was a time association with an ocean surge (11–14 April) and strong winds (12–20 knots on 12 and 13 April). Unfortunately, no environmental data such as levels of dissolved oxygen, suspended solids and toxic algae were available for the critical period 11–17 April inclusive and even some of that collected subsequently was not ideal. Clinical signs reported in the affected fish were that they were obviously distressed, swimming in a haphazard fashion at the surface and, in some instances, exhibiting gasping behaviour. On 15 and 16 April dead fish were reported to have large quantities of mucus flowing from their gills. This was not always the case with fish dying later in the episode. Most material available for histopathological examination was autolysed to some extent, but a small number of suitable samples revealed swelling of the respiratory epithelial cells and subepithelial oedema of the secondary lamellae. Possible aetiological factors responsible for the episode, either alone or in concert, were considered to be microalgal toxicosis, hypoxia, smothering by suspended solids and hydrogen sulphide toxicity. These are discussed in relation to the oceanography of Boston Bay and the unique anatomical and physiological characteristics of tuna.

Key words: brevetoxin, *Chattonella marina*, gill damage, microalgae, suspended solids, tuna

Fattening of captive southern bluefin tuna *Thunnus maccoyii* has been undertaken at Port Lincoln, mainly within Boston Bay, South Australia (Fig. 1 inset) since 1990 when a research and development program was established. Since that time development has been rapid and it was forecast that 2,800 t of tuna would have been produced in 1996 (Clarke, 1996).

Up until April 1996 most of the tuna were farmed in floating net pens in Boston Bay, west and south of Boston Island (Fig. 1), in a relatively shallow marine environment with relatively poor water exchanges of 9 days for winter and 24 days for summer (Bond, 1993). From April 1996 most pens have been moored outside Boston Bay at sites with more frequent water exchange. While being fattened the fish are fed on frozen baitfish, mainly pilchard *Sardinops sagax* at approximately 9% of bodyweight per day. This equates to approximately

250 t of wet fish per day and, as the food conversion ratio is approximately 17:1 (Smart, 1996), approximately 235 t of waste products of various types were added each day to the environment in Port Lincoln in early 1996. Up until 1996 losses of captive southern bluefin tuna were less than 15% per annum with much of this occurring around the time of capture (Rough *et al.*, 1997). The only infectious disease of note is a protozoal encephalitis which causes mortalities when the water temperature drops below 15°C (Munday *et al.*, 1997).

Over the period 12–13 April 1996 Port Lincoln was buffeted by strong winds of 12–20 knots (kn) and, commencing on 13 April, but mainly in the period 14–17 April there were massive mortalities of caged tuna (minimum 1,700 t) with lesser mortalities occurring into May 1996 (Clarke, 1996). Unfortunately, there was no functional, environmental monitoring equipment capable of measuring key water quality parameters in the region at the time. It was not until 18 April 1996 that environmental monitoring commenced (Clarke, 1996). Because of the lack of key environmental data diagnosis of

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Fig. 1. Maps showing location of Boston Bay (inset) and information on tuna mortalities as a percentage of the original stock.

the cause of the mortality proved difficult and this paper explores the differential diagnostic possibilities.

Material and Methods

Information and samples were obtained by one of the authors (BLM) during a visit to Port Lincoln on 19 and 20 April 1996. Additional information and samples

were made available by collaborators as acknowledged at the end of this paper.

Environmental sampling

The senior author flew over Boston Bay and Boston Island on the afternoon of 19 April 1996 prior to travelling by motor boat to affected farms to collect samples. On both occasions environmental conditions, as observed visually, were recorded.

Initial samples for microalgal examinations were collected on the afternoon (1600 to 1800 hours) of 19 April by vertical tows of a plankton net with a final mesh size of 100 µm. One sample was collected south of Boston Island just west of Bickers Island, three were collected inside the tuna cage line on the western side of Boston Island and one was collected approximately 0.5 km west of the above tuna cage line. These samples were preserved with 1.5% glutaraldehyde. Subsequently, on 23 April and 6 May 1996 a total of five unpreserved surface samples were collected.

The preserved samples were examined for the presence of toxic microalgae using standard techniques (Hallegraeff *et al.*, 1995). The unpreserved samples were also observed for toxic microalgae and cultured in GSe enriched seawater medium (Guillard, 1995).

Examination of affected tuna

Clinically affected fish were only observed at one farm on 19 April although descriptions were also obtained from observers present at the time of the major mortalities.

Only one affected tuna (body weight: approx. 20 kg) was necropsied by the senior author on 19 April. This fish was still twitching at the bottom of the netpen when collected by divers. A wide range of tissues, including gills were immediately placed in 10% formal saline for later histological examination. Other fish were necropsied by veterinarians at the Lincoln Veterinary Centre over the period 18 April to 14 May 1996 and preserved samples from these were provided to the authors for histology. All the preserved tissues were processed routinely to produce histological slides stained with haematoxylin and eosin and, also, alcian blue.

No samples for clinical pathology were collected on 19/20 April, but 10 samples from fish (average body weight: 25 kg) suffering continued mortality were collected on 14 May 1996 and analysed for a range of haematological and biochemical parameters by Veterinary Pathology Services, Adelaide.

Toxicological examinations

Livers were obtained from fish which had died on or about 15 April 1996 (two fish approx. 10 kg bodyweight), and two fish (approx. 26 kg bodyweight) which were harvested on 20 May 1996 from a group experiencing 1-2 mortalities per day. These were all analysed for brevetoxin (PbTx-3) using the synaptosome receptor binding assay (Trainer *et al.*, 1995).

Results

History

From discussions with people involved with tuna farming in Boston Bay the senior author was advised that the water had been turbid (visibility as little as 4m) for two to three months prior to the mortality episode. Surface water temperatures were about 21°C in the month before the mortality episode. Deeper readings varied from 16.6 to 22°C with the lower temperatures being recorded during early April. Little information was available for the period 14-16 April when temperatures at depth were 15.8-19°C.

At the commencement of the episode fish were described as distressed, swimming on the surface and, in some instances, showing a gasping type of behaviour. Excessive production of gill mucus was a common observation. There was a notable difference in mortalities between farms on the western side of Boston Island and those on the eastern side; 63-92% mortalities compared with 22-42%. (Fig. 1). There were no reported mortalities of wild fish.

Environmental conditions

From the air on 19 April the water in Boston Bay was turbid and a brownish slick was noted west of Bickers Island. The water east of Boston Island showed little turbidity. During collection of plankton samples the presence of large amounts of flocculent material (marine snow, Alldredge and Silver, 1988) was particularly noticeable.

The five glutaraldehyde-preserved phytoplankton net samples collected on 19 April all contained high concentrations of the raphidophyte flagellate *Chattonella marina* (Subrahmanyam) Hara et Chihara. The tear-drop shaped cells were 40-70 µm long, slightly distorted by the glutaraldehyde preservative, but could be readily recognised by having a large number of chloroplasts, producing a "raspberry" like cell appearance upon cell disintegration. A further unpreserved surface water sample collected at 1600 hrs on 23 April 1996 unam-

Fig. 2. *Chattonella marina* isolated from seawater samples.
a. and b. Typical organisms 30–70 $\mu\text{m} \times 20$ –30 μm at low and high powers.
c. Cell rounding up as a result of stress during examination under the microscope.

biguously confirmed the earlier identification and these cells were successfully isolated into unialgal culture in GSe enriched seawater medium (Fig. 2). The water sample contained approximately 1,000 *Chattonella* cells/l, but cell counts obtained by other investigators (Clarke, 1996) were as high as 66,000/l.

Other phytoplankton species present in the preserved samples were the dinoflagellates *Ceratium fusus*, *C. lineatum*, *C. tripos*, *Dinophysis caudata* (dominant), *D. cuneus*, *Gymnodinium* cf. *breve*. and *Gymnodinium catenatum*, the diatoms *Cerataulina*, *Coscinodiscus* and *Thalassiosira*, the coccolithophorid *Emiliania huxleyi* (common) and the raphidophyte *Heterosigma carterae* (= *akashiwo*). Of these species, *G. catenatum* (producer of paralytic shellfish poisons), *Gymnodinium* cf. *breve* and *H. cf. carterae* are known to have toxic potential (Hallegraeff, 1991), but compared to the abundance and size of *Chattonella*, these species were considered not to be of potential significance.

Affected fish

Affected fish on 19 April were observed swimming in a haphazard fashion rather than circling the cage. In one pen a fish was noticed to be “gasping” at the surface before sinking. This was the tuna that was subsequently necropsied. It was notable that this fish did not have excessive mucus on its gills, as had been reported for other affected fish, although another affected tuna

sampled by the Lincoln Veterinary Centre on 21 April also did not have excessive gill mucus. It is possible that these fish had exhausted their mucus cells as a result of earlier irritation during the episode.

Histologically the secondary lamellae of the gills of the fish sampled on 19 and 21 April showed marked epithelial swelling and variable lifting of the epithelium (sub epithelial oedema). (Fig. 3a). In some lamellae the nuclei of the swollen mucous cells were deeply basophilic. Congestion and haemorrhage were prominent in some gills. Mucus was not prominent between the lamellae except in some restricted areas, but some regions contained circular agglomerates of condensed mucus (Fig. 3c). There also was patchy mucus over the protective outer epithelium of the filaments which in some places appeared to block the fenestrations in the filaments. However, these observed mucus deposits can only be considered minimal because much of this material is lost during routine fixation (Powell *et al.*, 1992). A careful search was made of the mucus remaining attached to the gill and no unequivocal sediment particles were detected. A few metazoan parasites were detected in the filaments. Also, occasionally cells consistent with microalgae were detected between secondary lamellae (Fig. 3a). Apart from some oedema and desquamation of the skin epithelium over the scales, no remarkable changes were noted in organs apart from the gills. The samples collected on 14 May

1996 from fish being harvested exhibited minimal gill pathology, but there was a moderately-severe, multifocal, granulomatous myocarditis (Fig. 4) associated with

Fig. 4. Myocardium of tuna showing multifocal granulomata (arrows) H & E, bar = 50 µm.

an unidentified metazoan parasite.

The only abnormal clinical pathology results obtained from the blood samples collected on 14 May were that 6 out of 10 samples had elevated creatine kinase levels of 557 to 2153 U/l (mean 1295 U/l). These probably relate to the myocardial lesions mentioned above.

Toxicological examinations

The liver Pb Tx-3 equivalent values (triplicate analyses for replicate samples) were as follows; Fish 1 (died 15 April 1996): 24 and 36, Fish 2 (died 15 April 1996): 91 and 142, Fish 3 (harvested 20 May 1996): 45 and 51,

Fig. 3. Histology of normal and affected gills.

H & E, bar = 12.5 µm

- (a) Affected secondary lamellae exhibiting swollen epithelial cells (arrow heads), subepithelial oedema (arrow). Note presence of presumptive microalgal cell.
- (b) Normal secondary lamellae. Note thin epithelium (arrow head).
- (c) Affected gill showing accumulations of mucus (arrows).

Fish 4 (harvested 20 May 1996): 110 and 120 µg/100g liver tissue.

Discussion

As much critical data in relation to the period around the time of the mortality event is not available, arriving at a definitive diagnosis is difficult, if not impossible. Even so, likely hypotheses can be examined and ranked.

It is generally agreed that there was a strong time correlation between the strong winds and the mortality event. Petrusevics (1996) reported that the result would have been to produce a wave orbital speed under the seacages of about 8 cm/s. This would have been sufficient to resuspend fine silty sediments, such as under-cage organic fouling, but not fine to medium sands.

The likely differential diagnostic possibilities were adjudged to be: 1. Smothering by sediment, 2. Hydrogen sulphide toxicity, 3. Destratification of the water column bringing low-oxygen water to the surface, 4. Deoxygenation due to respiratory activity of bacteria and/or microalgae, 5. Inorganic toxicants, 6. Infectious diseases, 7. Toxic algal blooms.

1. Smothering by sediment

Superficially this was an attractive hypothesis, but was not supported by the facts as follows:

- (i) There was a 2 day delay between the time the storm impacted on the floor of the sea and when the major mortalities occurred. As mortalities due to suspended sediments occur due to clogging of the gills with particles and do not occur when the fish are able to prevent this clogging by "coughing" (Servizi and Martens, 1991), it is difficult to reconcile this delay with mortalities due to smothering by silt because the silt would gradually settle out of suspension. Also, it is pertinent that frank clogging of the gills with sediment was not seen by the authors or reported by any observer nor were significant quantities of silt detected histologically on the gills.
- (ii) Barry (1978) reported that yellowfin tuna, *Thunnus albacares* could withstand two weeks of exposure to turbid water of approximately 1.5 NTU. The turbidity readings for 18 and 19 April were mainly of 1.0 NTU with a very occasional reading of 2.0 NTU at 10-15 m. (Clarke and Paxinos, 1996).

2. Hydrogen sulphide toxicity

It is unlikely that hydrogen sulphide toxicity was

involved to any extent, if at all. Any reservoirs of hydrogen sulphide would have been released from the under-cage sediments when they were disturbed. This would have produced mortalities soon after the strong winds and not days later. Certainly, no sulphide was detected in water samples collected over the period 22-25 April 1996 (Christy *et al.*, 1996). Also, liver pathology typical of hydrogen sulphide poisoning (Kierner *et al.*, 1995) was not detected by the authors or fish pathologists reporting to the South Australian Government (Clarke, 1996).

3. Destratification of the water column

Petrusevics *et al.* (1993 abstract of oral presentation, CSIRO workshop) reported that significant stratification does not take place in Boston Bay. Also, mortalities would have occurred immediately after the strong winds and would not have continued as long as was observed.

4. Deoxygenation due to respiratory activity of bacteria and/or microalgae

It is quite possible that there was a bacterial bloom when the under-cage nutrients were resuspended in the water column and bacteria were able to utilize these and the relatively abundant oxygen for aerobic respiration. However, this would have been a short-lasting phenomenon because the nutrients and the bacteria would have then become diluted in the huge quantity of oxygenated water in Boston Bay. Therefore, it is possible that some of the early mortalities could have been due to hypoxia associated with a bacterial bloom, but not the ongoing mortalities. This is supported by Clarke and Paxinos (1996) who reported that dissolved oxygen during 18 and 19 April 1996 was in the range of 79.6-101.5% saturation.

Some localised reductions in dissolved oxygen could have occurred due to algal respiration during periods of low light intensity and, particularly, at depth. Such an occurrence was reported by Clarke and Paxinos (1996) at 0645 hours on 26 April 1996 at a depth of 16.7m where the dissolved oxygen was 65.4%. No turbidity reading was available for this site.

In summary, it is possible that some early mortalities were due to hypoxia, but not the majority. This is supported by the fact that the histological lesions were not typical of hypoxia (Ishimatsu *et al.*, 1996).

5. Inorganic toxicants

While the gill lesions could be consistent with toxicants such as dissolved copper or zinc (Mallatt, 1985) there is no known source of such at Port Lincoln. Also, there had been insufficient rain to flush any terrestrial sources of toxicants into the bay.

6. Infectious diseases

Apart from intercurrent parasitic infestations there was no histological evidence of infectious disease. Also, Clarke (1996) reported no evidence of bacterial or viral diseases.

7. Toxic algal blooms

Chattonella marina is toxic to fish by at least two mechanisms, the production of reactive oxygen radicals (Lee *et al.*, 1995) and production of ichthyotoxic brevetoxins (Khan *et al.*, 1995; Washburn *et al.*, 1994). The former probably produces direct damage to the sensitive gill tissues (Ishimatsu *et al.*, 1996) whereas the latter is absorbed from the water column (Lewis, 1992), presumably following rupture of the microalgal cells, and may produce cardiac irregularities (Endo *et al.*, 1992). The presence of both *C. marina* in the water column and high levels of brevetoxins in the livers of tuna in Boston Bay sampled at different times is indicative of substantial exposure of the fish to *C. marina* both before, and for at least a month after, the main mortality episode.

The possibility of the mortalities being a microalgal toxicosis due to *C. marina* was regarded by Clarke (1996) as unlikely because of the low numbers of cells (1,000–66,000/l) found in the water column in comparison with the reported lethal concentrations of 500,000/l or more for yellowtail *Seriola quinqueradiata* (Okaichi *et al.*, 1989). However, in this instance there would have been a synchronous growth burst due to the sudden addition of nutrients to the water column, resulting in the *C. marina* logically being at its most toxic at four days of growth at 17–18°C (c.f. two days at 26°C, Kawano *et al.*, 1996). This is in contrast to the older cultures [40,000 cells/ml, 1–2 weeks at 22°C (Tanaka *et al.*, 1994)] used in a number of the toxicity trials with yellowtail (Ishimatsu *et al.*, 1990, 1996) and often quoted in the literature. Also, the levels of *C. marina* in the water column were probably underestimated because marine snow, such as that which was present, has been shown to harbour up to 100% of the cells in the water column of simi-

lar microalgae (Alldredge and Silver, 1988).

Other supporting evidence for the involvement of toxic microalgae (*C. marina*) are the typical pathology (Ishimatsu *et al.*, 1996), the high gill area to bodyweight ratio (Hughes, 1984) and extremely high ventilation volume (Bushnell *et al.*, 1990) of tuna which would maximise exposure to the toxic effects of *C. marina* and the fact that the farmed tuna would have been depleted of endogenous antioxidants by the highly-oxidized baitfish used as feed (Munday *et al.*, 1997) and thus exquisitely sensitive to activated oxygen radicals.

The later, low-level mortalities could well have been due to the cardiotoxic effects of brevetoxins (Endo *et al.*, 1992) coupled with parasitic damage to the heart.

Conclusions

As indicated previously, we do not believe a definitive diagnosis is possible, but we do believe the weight of evidence points to a microalgal toxicosis due to *C. marina*, possibly accompanied by some deaths due to hypoxia produced by an initial bacterial bloom. For further clarification information is required on the susceptibility of tuna to toxic microalgae, or at least to activated oxygen radicals in the water, and the influence of endogenous antioxidants in protecting fish against *C. marina* blooms.

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