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Herpesvirus that caused epizootic mortality in 1995 and 1998 in pilchard, *Sardinops sagax neopilchardus* (Steindachner), in Australia is now endemic

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Abstract

Propagating epizootics due to *Pilchard herpesvirus* (PHV) occurred in the Australian population of pilchard, *Sardinops sagax neopilchardus* (Steindachner) (Clupeidae), in 1995 and 1998–99, with up to 60% losses. No mortality events have been evident in the ensuing 7 years, one reason for which could be that PHV is now endemic. During 2004, a survey was conducted to establish if PHV was present in pilchards in Australia. The pilchard is a highly active, pelagic schooling fish which is found in subpopulations, creating difficulties for the conduct of surveys. It occurs in Australian coastal waters and embayments below about 25 °S latitude, feeds on plankton and is predated by birds, mammals and larger fish. It reaches sexual maturity at 2 years of age, spawns at sea, enters embayments when about 5 months old and returns to sea when about 1 year old. It may live for 6–9 years, reaching a maximum length of 200 mm. It forms schools and may travel up to 30 km per day. Pilchards aggregate in mobile shoals of fish containing large highly mobile schools, which interact randomly and exchange individuals. Four subpopulations were defined for the purposes of this survey based on differences in biological characteristics: south-eastern Queensland/northern New South Wales (NSW), Victoria/South Australia (SA), south coast Western Australia (SWA) and west coast Western Australia (WWA). Specimens were obtained from the catch of commercial fishermen using random sampling where possible. Polymerase chain reaction (PCR) for the detection of PHV was performed after appraising the suitability of all available tests according to their impact on sample size requirements, total survey costs and logistical constraints. In the analysis, estimates of true prevalence (TP) of infection and 95% confidence limits were adjusted from the apparent prevalence estimates provided by PCR results. Percentage TP of PHV and corresponding 95% confidence intervals for the four subpopulations: NSW, SA, SWA and WWA were thus estimated as 0 (0–1.5), 31 (22–43), 42 (31–55) and 29 (20–41), respectively. PHV is now endemic in Australian populations of pilchard. Implications of the findings for fisheries management are discussed.

Keywords: ecosystem health, herpesvirus, PCR, pilchard, *Sardinops sagax*, survey.

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Introduction

The Australasian pilchard, *Sardinops sagax neopilchardus* (Steindachner) (Clupeidae), is an abundant and widespread resident of coastal waters and embayments below about 25 °S latitude (Neira, Sporcic & Longmore 1999; Ward & Staunton-Smith 2002). The commercial fishery in Australia took on average about 10 000 tonnes p.a. in the 1990s (Fletcher 2001), but this subsequently increased to 40 000 tonnes in 2001 (AQIS 2003). This species also occurs in New Zealand coastal

Figure 1 Dead pilchards on a beach in Western Australia during the mortality event in 1998.

waters while closely related subspecies occur in Japanese waters and along the Pacific coasts of South America and South Africa. A pelagic fish, its distribution is probably limited to waters of the continental shelf to depths of 100–200 m and water temperatures of 12–20 °C (Fletcher 1990). Pilchards feed on plankton and are in turn predated by fish, birds and mammals. Due to the difficulty of study, little is known about the ecology of pilchards < 2 years of age (Fletcher 2001).

In 1995, a massive epizootic occurred in pilchards. It commenced in May in South Australian waters and spread bidirectionally until November over 6000 km, throughout the range of the species in Australian waters, and then spread to New Zealand. A similar event occurred in Australia between October 1998 and April 1999, but there have been no further outbreaks. The disease was characterized by severe branchitis and was attributed to a herpesvirus, *Pilchard herpesvirus* (PHV), on clinical, pathological and epidemiological grounds (Hyatt, Hine, Jones, Whittington, Kearns, Wise, Crane & Williams 1997; Jones, Hyatt, Hine, Whittington, Griffin & Bax 1997; Whittington, Jones, Hine & Hyatt 1997). Mortality was severe, with dead fish washed onto beaches in vast numbers (Figs 1 & 2) and accumulating also on the sea floor (Whittington *et al.* 1997; Gaughan, Mitchell & Blight 2000). Estimates of mortality rates ranged as high as 75% (Gaughan *et al.* 2000). Consequently there were measurable secondary impacts in piscivorous species including penguins, which experienced increased mortality rates and failed to breed due to food shortage (Dann, Norman, Cullen, Neira & Chiaradia 2000). The proportion of pilchard in the diet of the Australasian gannet dropped from 50% to 5% as they switched to other prey items (Bunce & Norman 2000). As alternate prey were of lower calorific value gannets may also have had reduced reproductive success (Bunce & Norman 2000). The scale of the impact on ecosystem health and duration of impact has not been quantified. However, the importance of these disease outbreaks on food webs has been highlighted (Bunce & Norman 2000). For this reason greater knowledge of the epidemiology of PHV is important, in particular its current distribution and abundance in pilchard populations.

Figure 2 Gross signs of *Pilchard herpesvirus* infection. Pilchards that died in extremis with mouth open and opercular covers adducted, New South Wales, 1995.

Herpesviruses have general properties including host specificity and establishment of persistent or lifelong infection. Latent infections may periodically breakdown in individuals, resulting in clinical disease. For these reasons the outbreaks of PHV in Australasia could have been due either to introduction of the virus into a naïve population, or to reactivation of latent infection within the popula-

tion. Consistent with the first hypothesis was the observation that both outbreaks began in South Australia near where thousands of tonnes of pilchard imported from America were fed to sea-ranched tuna. Mathematical modelling suggested that in both 1995 and 1998–99 the virus originated from a point source, travelled without the need for vectors in a host-density independent manner, and had an incubation time of several days, all of which fitted the observed pattern of disease spread and the pathogenesis of the lesions in gills (Whittington *et al.* 1997; Murray, O'Callaghan & Jones 2000, 2001a,b, 2003). Reversion of latent PHV infection seems a much less plausible cause of the epizootic because the disease had not been reported previously in Australian pilchards and there had been no discernable environmental events immediately prior to the epizootic that could trigger reversion on such a large scale and over such a wide area (such as cold water upwellings, unusual currents or toxic phytoplankton bloom) (Griffin, Thompson, Bax, Bradford & Hallegraeff 1997). Regardless of the causes of the initial outbreak, the absence of any further outbreaks since 1998–99 suggests that the virus may now be endemic in Australia, either in latent form or as a cause of unrecognized low-grade disease in an immune population. Here, we describe a survey that was undertaken to determine whether PHV is still present within Australian pilchard populations.

Materials and methods

Survey population and design

A literature review was conducted to define the population to be surveyed, constraints and opportunity for survey (Table 1, see Results). The sampling frame was restricted to pilchards in the four defined subpopulations: south-eastern Queensland/northern New South Wales (NSW), Victoria/South Australia (SA), south coast Western Australia (SWA), west coast Western Australia (WWA) (Fig. 3, see Results). Based on the ecology of PHV infection including observations made during the initial outbreak and computer modelling, the biology of pilchards and for reasons of practicality in conducting a survey, it was assumed that if the virus was present it would be found in at least 10% of adult fish and would be uniformly distributed among shoals of fish within a subpopulation and among schools within shoals.

Table 1 Biology of the Australasian pilchard, *Sardinops sagax neopilchardus*

Distribution	Coastal waters and embayments below about 25 °S latitude
Habitat	Pelagic, continental shelf to 200 m depth and water temperatures 12–20 °C
Feed	Plankton
Predators	Fish, birds and mammals
Breeding	Sexual maturity 2 years, spawn at sea, juveniles enter bays at 5 months, return to sea when 1+ years old
Lifespan	6–9 years
Size	200 mm max
Migration	1000 km at 30 km per day possible
Behaviour	Schooling, maintain constant spatial separation

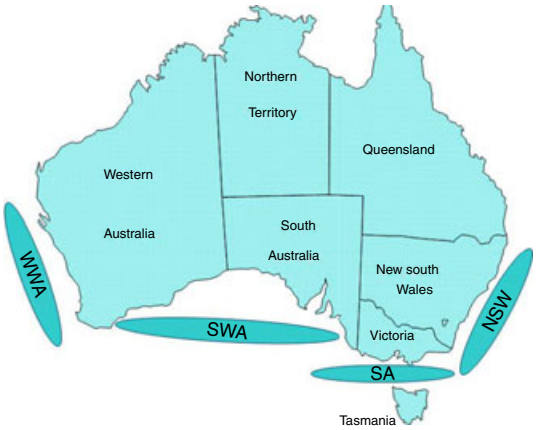

Figure 3 Subpopulations of *Sardinops sagax neopilchardus* in Australia. NSW, south-eastern Queensland/northern New South Wales; SA, Victoria/South Australia; SWA, south coast Western Australia; WWA, west coast Western Australia.

The number of fish sampled per subpopulation was estimated using the Freecalc software program (Cameron 2002). This derives the sample size required for designating the true infection status of a group (subpopulation) by taking into account the likely minimum prevalence (above), sampling variation and the imperfect performance of diagnostic tests as defined by sensitivity and specificity. The available tests are histopathology, electron microscopy, polymerase chain reaction (PCR) and *in-situ* hybridization of gill tissues. There are no data on sensitivity or specificity for these tests, so conservative estimates were input into Freecalc with the output used to guide the selection of tests for PHV infection and the required sample size (Table 2). PCR was the only practical test method based on these assumptions, cost of tests and project budget. Two hundred fish from each subpopulation were

Table 2 Sample size required for detection of PHV in each subpopulation

Test applied to gill tissue	Assumed sensitivity (%)	Assumed specificity (%)	Sample size required	Threshold no. of reactors
Electron microscopy	20	95	2599	148
Histopathology	50	90	711	84
In situ hybridization	30	98	452	14
PCR	50	98	191	7

The threshold number of reactors is the minimum number of positive tests required for a population to be designated as truly infected.
Assumes population > 10 000, expected prevalence 10%, confidence level 95%, and most likely values for sensitivity and specificity.

examined. Sample sizes were not adjusted for the additional source of variation due to the clustered nature of the population, because no data exist to estimate the magnitude of the design effect for this setting.

Sampling

Netting techniques used for harvesting pilchards are purpose designed for the capture of schools of adult fish and these are also the age class of interest for detection of PHV. Individual fish were selected for testing in a way that best approximated random sampling. Specimens were collected with the assumption that schools of fish targeted at any time by commercial purse-seine fishermen were representative of the subpopulation from which they were caught. Sampling was purposive in each region in that efforts were centred on those harvesting operations with the best access to each subpopulation. The timing of sampling was based on convenience according to the availability of personnel, resources and the cooperation of fishermen, but was always confined to those months in which outbreaks of PHV infection had been observed previously.

Fishermen collaborated in the collection of specimens after receiving written or verbal instruction from researchers. Fish were drawn to the boat within the net and packaged in boxes with ice. During the haul the size of the catch was estimated (number of boxes) as was the approximate number of fish per box. Systematic random sampling was used to select the particular boxes from which individual fish would be collected, with a dice rolled to determine the number of the first box from which samples were extracted. Samples were subsequently extracted from every *n*th box until the required number of fish was collected, with *n* defined so that sampling occurred across the entire catch. When sampling from each box a spatial sampling plan was employed by visually discerning 27 equal-size compartments with individual fish extracted from those compartments according to a list of random codes identifying the three-dimensional location of the compartment. This method was applied in each subpopulation except NSW where only fresh frozen material from a cold store was available. The management of the cold store selected a box of frozen fish from others based on criteria emphasizing convenience. The box was then sent to the researchers who selected the required number of fish approximately randomly.

Analytical methods for PHV

Nucleotide sequence data for the genome of PHV were obtained from amplicons produced in PCR assays using primers designed from conserved regions of the genomes of other finfish herpesviruses (Crockford, Jones, Crane & Wilcox 2005). Viral template was obtained from infected pilchard gill samples. Primers for a real-time PCR assay for PHV were designed based on actual sequence data for PHV. Samples for detection of PHV in the survey were prepared from fresh gill tissues. Methods for real-time PCR and sample preparation were as described (Crockford *et al.* 2005; Crockford, Jones, McColl & Whittington 2007).

Estimation of true prevalence

Apparent prevalence (AP, the proportion of fish that are test positive) was used to estimate true prevalence (TP, the proportion of fish truly infected with PHV) using the formula TP = (AP + specificity − 1)/(sensitivity + specificity − 1) (Thrusfield 1995) and the sensitivity and specificity estimates for PCR shown in Table 2. Exact binomial confidence limits for AP were calculated using the relationship between the binomial and inverse-beta probability distributions (Gelman, Carlin, Stern

& Rubin 1995), while confidence limits for TP were calculated by using the upper and lower 95% confidence limit estimates for AP in the above formula (Thrusfield 1995). We also modelled the impact of uncertainty in estimates of sensitivity and specificity by allowing each test parameter to vary by about 10% from their assumed values and recording the effect on TP and its 95% confidence limits.

Results

Review of pilchard biology and survey considerations

Pilchards are highly active, pelagic schooling fish which creates difficulties for the conduct of surveys. However, pilchard larvae up to 20 mm can be sampled using plankton nets with 0.3–0.5 mm mesh, with larvae of 30 mm rarely captured; adults >120 mm can be captured using purse seine nets, with most surveys run in conjunction with commercial fishing activities (Fletcher 1990). Key attributes of the biology of *S. sagax neopilchardus* as they influenced design, implementation and interpretation of the survey are summarized in Table 1 and will be discussed in more detail below.

In south-east Queensland, at the northern limit of their range, pilchards are most abundant during the spawning season in winter, when water temperatures are <23 °C (Ward & Staunton-Smith 2002). Seasonal catch data from south-east Queensland are consistent with there being a seasonal migration of schools from northern New South Wales in winter (Ward & Staunton-Smith 2002), consistent with anecdotal reports (cited by Fletcher, Jones, Pearce & Hosja 1997). There are similar anecdotal reports of migration from east to west in Western Australian waters (Fletcher *et al.* 1997). Migrations of this species for distances of over 1000 km are recorded from capture-mark-release work in South Africa and there is consensus from several studies that migration rates of up to 30 km per day are plausible based on swimming velocity (reviewed in Fletcher *et al.* 1997). During migration shoals (see below) remain intact, one such shoal of South African pilchards reportedly migrated 27 nautical miles (50 km) in a day (Cram & Hampton cited by Blaxter & Hunter 1982). Furthermore, eggs and larvae can be moved over great distances by currents. For example, the Leeuwin current moves larvae 160 km east from Albany, Western Australia while westerly movements can occur during summer (Fletcher 2001).

The mortality wave observed in both the 1995 and 1998–99 outbreaks has been explained using conventional stage-transition theory for propagation of infectious disease and the models created have accurately reproduced the observed patterns of spread, including a recurrent mortality near the origin prior to wave-like propagation (Murray *et al.* 2003). The former phenomenon presumably corresponds to a period where viral loads are building in a school to reach levels sufficient for efficient dispersion to other schools. These models assume random contact between schools of fish and exchange of infected individuals between schools (Murray *et al.* 2001a, 2003). An assumption implicit in the wave spread model (Murray *et al.* 2003) is that there is a semi-continuous population of pilchards spanning the geographic extent of the disease event. The models do not rely on migration of fish or mammalian or avian vectors. Modelling also suggested that the infection rate was very high, approaching 100%, and that it exceeded the mortality rate (Murray *et al.* 2003).

For the purposes of this survey, it was assumed that pilchard populations consist of mobile shoals of fish that contain large highly mobile schools (Fig. 4). This was based on the modelling data above and biological observations. Schooling is a behavioural trait, which develops in the post-larval stage and increases with age (Blaxter & Hunter 1982). Schools mostly consist of similar sized fish; schools with larger or smaller sized fish may be found nearby (Fletcher *et al.* 1997). Differences in swimming velocity between small and large fish account for the tendency of similar sized fish to remain together (Blaxter & Hunter 1982). The schools interact randomly and exchange individuals. Within a shoal, schools may coalesce, separate and

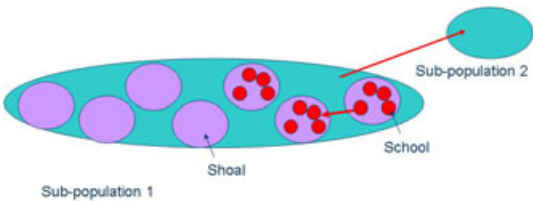

Figure 4 Model of the pilchard population. Subpopulations of pilchards contain shoals which consist of schools; individual fish escape from one school to another (short arrow) during close contact of schools and shoals exchange individuals both within and between subpopulations (long arrow).

reform during the day. Schooling behaviour may also be linked to light intensity, with schools disaggregating at night (Blaxter & Hunter 1982). Mixed species clupeoid schools with one dominant species have been observed in California (Blaxter & Hunter 1982). Schooling clupeoids tend to maintain constant spatial separation, with mean nearest neighbour distances of about one body length for most species, creating densities of 115–366 fish m^{-3} (Blaxter & Hunter 1982).

Pilchards are sexually mature from about 2 years of age at about 120 mm LCF and may live for 6–9 years (Blackburn 1949; Fletcher *et al.* 1997). In Western Australia most of the commercial catch consists of fish 140–160 mm (2–5 years old) and the maximum length reached is 200 mm (8 years old) (Fletcher 2001). Spawning occurs at various times of year depending on location (for example, April to July in the Great Australian Bight; both July and December–January at Albany), but is consistent at each location (Fletcher *et al.* 1997). Pilchards probably spawn at sea, with juveniles entering bays and inlets from about 5 months of age, heading back to sea the following year when 1+ years old (Blackburn 1949; Neira *et al.* 1999). However, reproductive data for many regions are scarce despite significant research effort (Gaughan 2001).

There appear to be several populations of pilchards in Australian waters. The evidence for this includes differences in spawning times, age structure, morphometrics, alloenzyme studies, and studies of the trace element and isotope content of otoliths (reviewed in Fletcher *et al.* 1997). Work conducted in the 1950s based on spawning time and vertebral counts (Blackburn 1951) suggested that there were three distinct populations of pilchard in Australian waters, an eastern group (New South Wales), a south-eastern group (Victoria) and a south-western group (Western Australia). This concept is still believed to be correct but more recently another group has been added (WWA) (Syahailatua 1992 cited by Fletcher *et al.* 1997). Within Western Australian waters, there appear to be two separate populations based on spawning times (west coast and south coast), but two to three separate populations of adults exist along the south coast. The stocks vary in position and juveniles may flow from one area to another from a common nursery site, which has not been identified (Fletcher *et al.* 1997). The movement of eggs and larvae between sites leads to genetic mixing (Fletcher 2001), even though adult populations may be relatively separate. For the purposes of management of commercial fisheries, the Western Australian Department of Fisheries recognizes four distinct pilchard management zones which reflect these populations of adult pilchards (Cochrane 1999). These are the West Coast Purse Seine Fishery (Fremantle) and the South Coast Purse Seine Fishery (Albany, Bremer Bay and Esperance).

Four subpopulations of pilchard were defined for the purposes of this survey: NSW, SA, SWA and WWA (Fig. 3). This was a conservative approach given the likely mixing that occurs and the prior observed spread of PHV that involved all subpopulations.

Prevalence of PHV

The overall AP of PHV infection in the Australian pilchard population based on PCR was 14% but the NSW subpopulation had lower prevalence than the other subpopulations (Table 3). As the number of positive samples from NSW did not exceed the cut-point number of reactors (Table 2) this subpopulation may not in fact be infected with PHV. True prevalence ranged between 25% and 42% for the three (overtly) infected subpopulations with the highest upper confidence limit being 55% for SWA.

The estimates of true prevalence were moderately sensitive to assumptions of test sensitivity and specificity (Table 4). For example, for SWA the most extreme values for the upper confidence limit were 47–64% when sensitivity and specificity were allowed to vary in the range 0.44–0.56 and 0.965–

Table 3 Estimated true and apparent prevalence of PHV infection in Australian pilchards calculated assuming that PHV-infected fish are randomly distributed within subpopulations and not clustered in particular shoals or schools

	Apparent prevalence (%)		True prevalence (%)	
Subpopulation	Point estimate	95% confidence limits	Point estimate	95% confidence limits
NSW	0.5	0.12–2.7	0	0–1.5
SA	17	12–23	31	22–43
SWA	22	17–28	42	31–55
WWA	16	12–22	29	20–41
Overall	14	10–20	25	16–37

NSW, south-eastern Queensland/northern New South Wales; SA, Victoria/South Australia; SWA, south coast Western Australia; WWA, west coast Western Australia.

Table 4 Sensitivity of estimates of true prevalence of PHV to variation in the performance of the PCR test for virus in pilchard gill tissue

	Upper 95% confidence limits Test specificity/ sensitivity		Lower 95% confidence limits Test specificity/ sensitivity	
Subpopulation	99.5/44%	96.5/56%	99.5/44%	96.5/56%
NSW				
SA	0.51	0.37	0.27	0.17
SWA	0.64	0.47	0.38	0.25
WWA	0.49	0.35	0.25	0.15
Overall	0.44	0.30	0.22	0.12

The table shows the most extreme estimates for 95% upper and lower confidence limits obtained with test specificity in the range 96.5–99.5% and test sensitivity in the range 44.0–56.0%.
NSW, south-eastern Queensland/northern New South Wales; SA, Victoria/South Australia; SWA, south coast Western Australia; WWA, west coast Western Australia.

0.995, respectively. The corresponding range for the lower confidence limit for this subpopulation was 25–38%.

Discussion

Surveys of pilchards have been hampered by the wide geographic distribution, high mobility, high capacity for net avoidance, differential catchability of sex and age classes and temporal variation in distribution and behaviour of the species (Ward, Westlake, McLeay & Jones 1999). Observations and modelling of the spread of PHV disease in the 1995 and 1998–99 epizootics suggested that there is a single semi-continuous Australian pilchard population with direct contact between shoals of adult fish and between schools within shoals sufficient to enable wave-like propagation of infection following initiation from a point source and after a period of local build up of viral loads (Murray *et al.* 2000). Nevertheless, we assumed that the population consisted of four separate subpopulations for the purposes of this survey and sampled each separately, which was a conservative approach. We conducted a census of subpopulations, and within each a convenience sample of catchable schools of fish was taken by commercial fishermen. Random sampling within the commercial fishing catch was then applied by the fishermen in three of the four subpopulations and convenience sampling in the other. Interpretation of the results was based on an assumption that PHV is uniformly distributed and not clustered among schools of fish, where a school is the aggregate of individual fish susceptible to purse-seine netting. This would appear to be a reasonable assumption based on modelling of the spread of PHV, observations of propagating mortality in both 1995 and 1998–99 and schooling behaviour of pilchards (see Results). It would also mitigate against violations of the assumption of random sampling if fishermen did not follow the protocol for sampling.

The results from the NSW subpopulation were different from the others. This group of fish was not sampled at random which means that there is more uncertainty about true prevalence and its confidence limits; the possibility remains that infection is clustered elsewhere within the subpopulation.

In the absence of accurate estimates of test sensitivity and specificity and our ability to implement strict random sampling within a multi-stage survey design there is uncertainty about the true prevalence of PHV. However, even after allowing for uncertainty in test performance, it is clear that infection is definitely present in the SA, SWA and WWA subpopulations, where a substantial proportion of pilchards are infected. In the SA subpopulation, which had the highest point prevalence, the best estimate would be that between 25% and 64% of fish are infected; these values correspond to the most extreme values for lower and upper confidence limits, respectively. Some caution is needed when interpreting the confidence limits because of the multi-stage nature of the sampling within each subpopulation and the effect of possible clustering, which we assumed not to be present based on biological data and modelling of the epizootic. Effectively this type of sampling introduces a source of variance into the data that cannot be accounted for by methods available to us for estimating confidence limits in this study. The practical consequence is that the widths of the confidence intervals are likely to be underestimated (Wang, Yu & Lin 1997). In any case, it would not be practical to undertake multi-stage sampling of marine pelagic fish at the level of shoal and school. This is an issue common to all marine surveys.

Based on the results of the survey it can be concluded that PHV is now endemic in *S. sagax neopilchardus* in Australian waters. Together with the absence of epizootic mortality since 1999 this is an important finding which reinforces the hypothesis that PHV was introduced to a naïve population in 1995 and again in 1998–99 when it became established. The fish exposed in 1995 are now all dead, either as a result of infection with PHV or

due to lifespan (Table 1) as are most of the fish alive in 1998. The PHV positive fish sampled in 2004, all of which were adult, were therefore recently exposed to the virus and may be immune. The modelling by Murray *et al.* (2000, 2001a,b, 2003) indicates that the epizootic wave will not develop in the absence of a point source and a naïve population. This may explain why no further epizootics have been seen despite the presence of the virus in Australian waters. It is unknown whether PHV currently induces pathology of gills or mortality, or whether individual animal and herd immunity is sufficient to limit the severity of PHV to subclinical infection. The NSW subpopulation has a low prevalence of PHV or may be uninfected. Additional surveys will be required to confirm this, and they need to include pilchard schools in southern NSW and Victorian waters. It would also be interesting to test New Zealand populations, where an epizootic was observed in 1995.

The findings of this study have implications for fisheries managers. Subject to the results of additional surveys, it may be beneficial to manage anthropogenic influences to limit contact between the NSW and SA subpopulations. These influences would include translocation of southern pilchards in the bait industry.

Current international translocations of baitfish including pilchards clearly place naïve populations at risk of acquiring pathogens to which there is no prior exposure and immunity. In addition to immediate economic costs associated with loss of a fishery there are potentially larger ecological consequences as food chains are impacted and predator prey relations change.

There have been no surveys for PHV in other populations of *S. sagax neopilchardus* but they would be worthwhile. We would predict endemicity of PHV in populations in the Americas and possibly elsewhere to explain the epizootics in Australia and New Zealand, which were probably initiated by translocation of bait fish (Whittington *et al.* 1997). Such surveys would be relatively easy to complete given the availability of a sensitive PCR assay and this, if positive, then enables comparisons of viral nucleic acid sequence to assist understanding of the mechanisms of spread of this virus.

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