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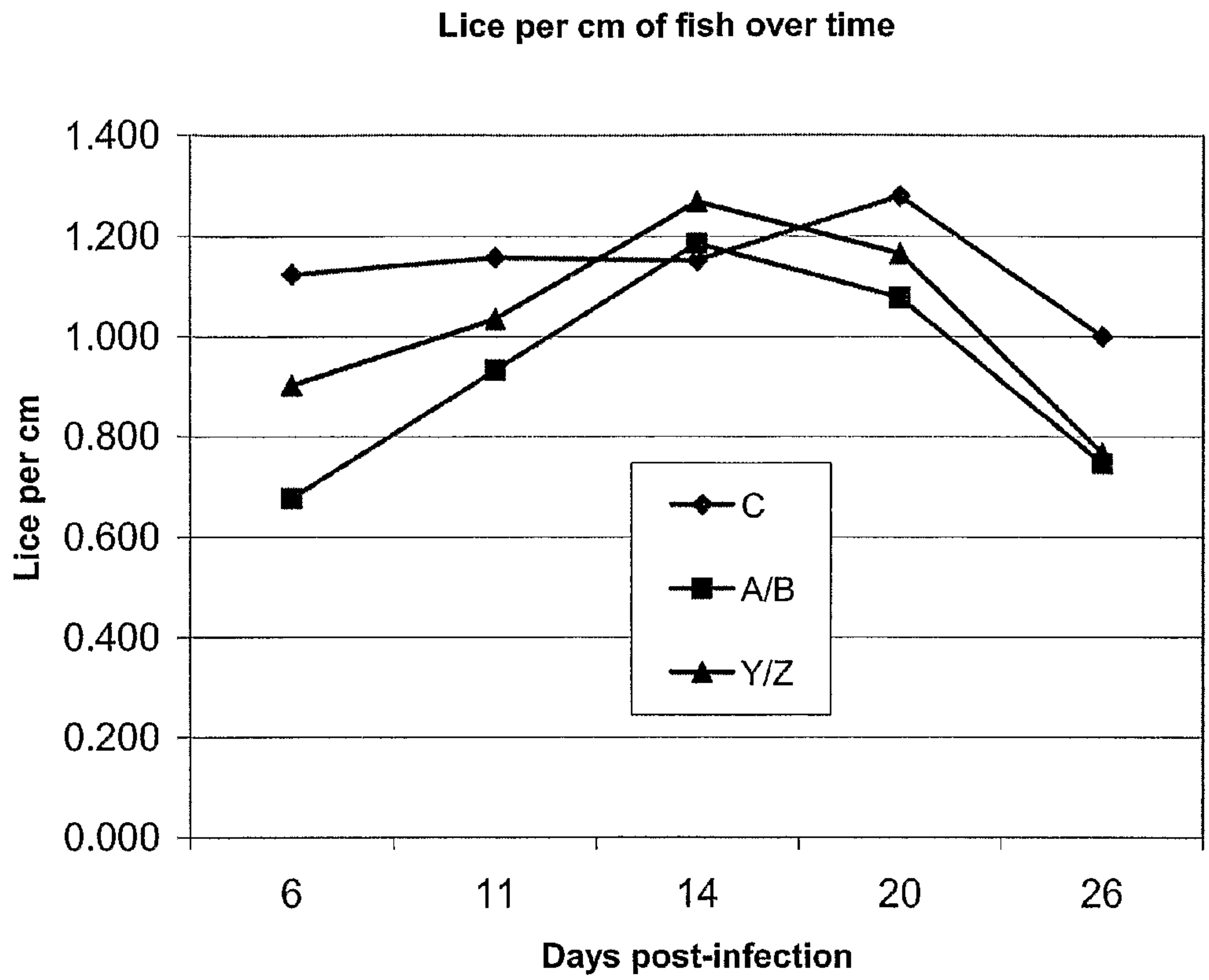
(54) Title: RECOMBINANT VACCINES AGAINST CALIGID COPEPODS (SEA LICE) AND ANTIGEN SEQUENCES THEREOF




(57) Abrégé/Abstract:
Molecular targets and vaccines against them in the treatment of sea lice infection of fish are provided, particularly caligid copepods. Vaccines targeted to L. salmonistrypsin are shown to reduce the quantity of sea lice present in challenged salmon from day 14 p.i. onward. Additional and novel molecular targets for vaccines are also provided.

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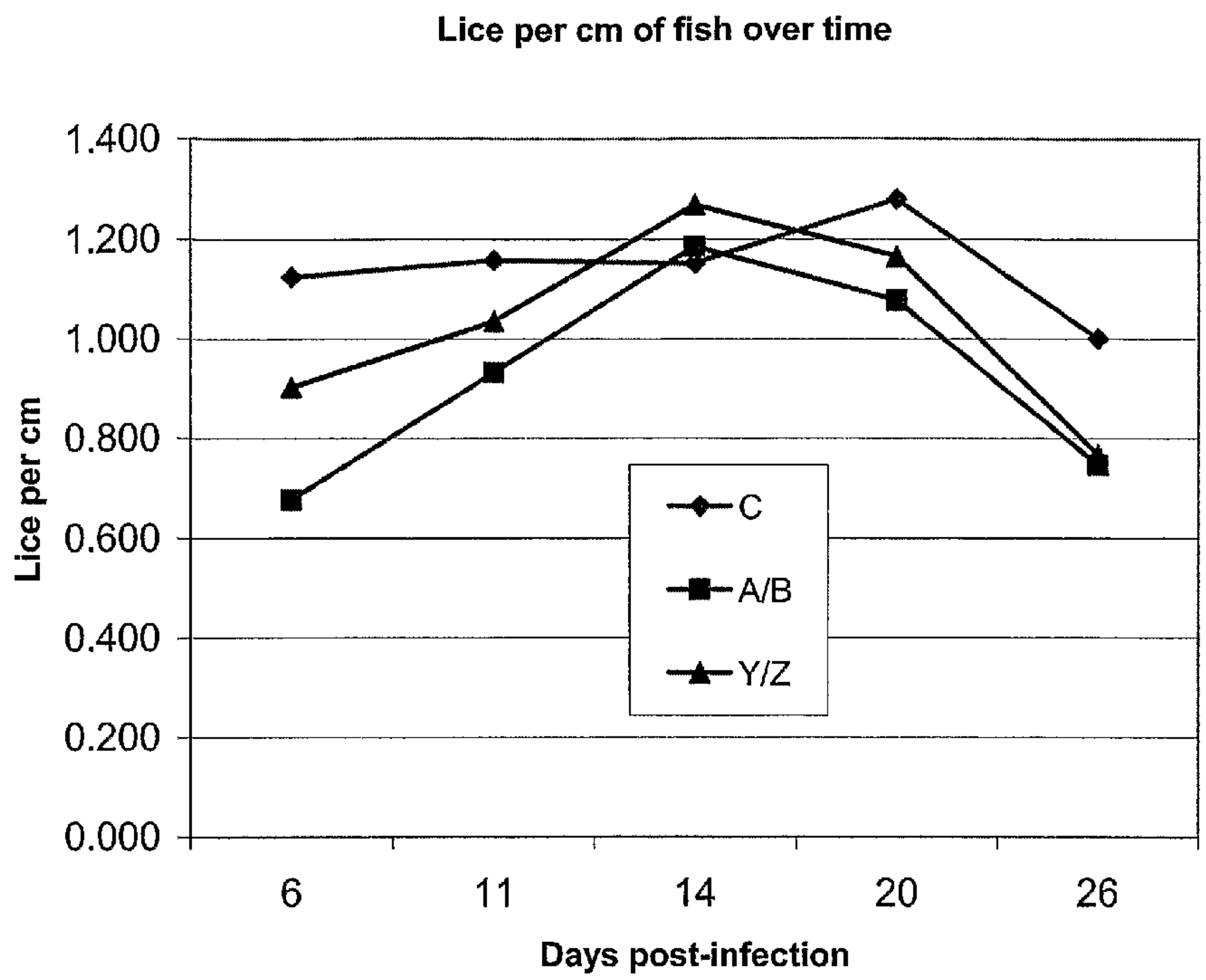


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# JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.**

**THIS IS VOLUME __1___ OF __2___**

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# RECOMBINANT VACCINES AGAINST CALIGID COPEPODS (SEA LICE) AND ANTIGEN SEQUENCES THEREOF

## FIELD OF THE INVENTION

The present invention relates generally to salmon vaccines. More particularly, the present invention relates to vaccines against parasitic caligid copepods (sea lice) and antigen sequences thereof.

## BACKGROUND OF THE INVENTION

A number of closely related species of parasitic copepods in the family *Caligidae* (caligid copepods) infect and cause disease in cultured fish. Collectively, these species are referred to as sea lice. There are three major genera of sea lice: *Pseudocaligus, Caligus* and *Lepeophtheirus*. With respect to salmonid production throughout the northern hemisphere, one species, the salmon louse (*Lepeophtheirus salmonis*), is responsible for most disease outbreaks on farmed salmonids. This parasite is responsible for indirect and direct losses in aquaculture in excess of US $100 million annually (Johnson, S.C., *et al.*, Zool Studies 43: 8-19, 2004). All developmental stages of sea lice, which are attached to the host, feed on host mucus, skin and blood. The attachment and feeding activities of sea lice result in lesions that vary in their nature and severity depending upon: the species of sea lice, their abundance, the developmental stages present and the species of the host (Johnson, S.C. et al., "Interactions between sea lice and their hosts". In: *Host-Parasite Interactions*. Editors: G. Wiegertjes and G. Flik, Garland Science/Bios Science Publications, 2004, pp. 131-160). In the southern hemisphere, *Caligus rogercresseyi,* is the primary caligid affecting the salmon farming industry in Chile (González, L. and Carvajal, J. Aquaculture 220: 101-117, 2003).

Caligid copepods have direct life cycles consisting of two free-living planktonic nauplius stages, one free-swimming infectious copepodid stage, four to six attached chalimus stages, one or two preadult stages, and one adult stage (Kabata, Z. , Book 1: Crustacea as enemies of fishes. In: *Diseases of Fishes.*, Editors: Snieszko, S.F. and Axelrod, H.R.; New York, T.F.H. Publications, 1970, p. 171). Each of these developmental stages is separated by a moult. Once the adult stage is reached caligid copepods do not undergo additional moults. In the case of *L. salmonis,* eggs hatch into the free-swimming first *nauplius* stage, which is followed by a second *nauplius* stage, and then the infectious *copepodid* stage. Once the copepodid locates a suitable host fish it continues its development through four *chalimus* stages, first and second preadult stages, and then a final adult stage (Schram, T.A. "Supplemental descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae)". In: *Pathogens of Wild and Farmed Fish: Sea Lice*. Editors: Boxshall, G.A. and Defaye, D., 1993, pp. 30-50). The moults are characterized by gradual changes as the animal grows and undertakes physical modifications that enable it to live as a free-roaming parasite, feeding and breeding on the surface of the fish.

Caligid copepods (sea lice) feed on the mucus, skin and blood of their hosts leading to lesions that vary in severity based on the developmental stage(s) of the copepods present, the number of copepods present, their site(s) of attachment and the species of host. In situations of severe disease, such as is seen in Atlantic salmon (*Salmo salar*) when infected by high numbers of *L. salmonis,* extensive areas of skin erosion and hemorrhaging on the head and back, and a distinct area of erosion and sub-epidermal hemorrhage in the perianal region can be seen (Grimnes, A. *et al.* J Fish Biol 48: 1179-1194, 1996). Sea lice can cause physiological changes in their hosts including the development of a stress response, reduced immune function, osmoregulatory failure and death if untreated (Johnson et al., *supra*).

There are several management strategies that have been used for reducing the intensity of caligid copepod (sea lice) infestations. These include: fallowing of sites prior to restocking, year class separation and selection of farm sites to avoid areas where there are high densities of wild hosts or other environmental conditions suitable for sea lice establishment (Pike, A.W. *et al.* Adv Parasitol 44: 233-337, 1999). Although the use of these strategies can in some cases lessen sea lice infection rates, their use individually or in combination has not been effective in eliminating infection.

A variety of chemicals and drugs have been used to control sea lice. These chemicals were designed for the control of terrestrial pests and parasites of plants and domestic animals. They include compounds such as hydrogen peroxide, organophosphates (e.g., dichlorvos and azamethiphos), ivermectin (and related compounds such as emamectin benzoate), insect molting regulators and pyrethrins (MacKinnon, B.M., World Aquaculture 28: 5-10, 1997; Stone J., *et al.*, J Fish Dis 22: 261-270, 1999). Sea lice treatments can be classified into those that are administered by bath (e.g. organophosphates, pyrethrins) and those administered orally (e.g. ivermectin). Bath treatments for sea lice control are difficult, expensive to apply and can have significant effects of fish growth following treatments (MacKinnon, *supra*). Chemicals used in bath treatments are not necessarily effective against all of the stages of sea lice found on fish. At present the use of oral treatments such as SLICE® (emamectin benzoate) is predominant in the salmonid industry. Unlike chemicals administered as bath treatments SLICE® does provide short-term protection against re-infection. This treatment although easier to apply than bath treatments is still expensive and, like bath treatments, requires a withdrawal period before animals can be slaughtered for human consumption (Stone, *supra*). As seen in terrestrial pest and parasites there is evidence for the development of resistance in *L. salmonis* to some of these treatments, especially in frequently-treated populations (Denholm, I., Pest Manag Sci 58: 528-536, 2002). To reduce the costs associated with sea lice treatments and to eliminate environmental risks associated with these treatments new methods of sea lice control such as vaccines are needed.

A characteristic feature of attachment and feeding sites of caligid copepods on many of their hosts is a lack of a host immune response (Johnson *et al.*, supra; Jones, M.W., *et al.*, J Fish Dis 13: 303-310, 1990; Jónsdóttir, H., *et al.*, J Fish Dis 15: 521-527, 1992). This lack of an immune response is similar to that reported for other arthropod parasites such as ticks on terrestrial animals. In those instances suppression of the host immune response is due to the production of immunomodulatory substances by the parasite (Wikel, S. K., *et al.*, "Arthropod modulation of host immune responses". In *The Immunology of Host-Ectoparasitic Arthropod Relationships*. Editors: Wikel, S. K., CAB Int., 1996, pp. 107-130). These substances are being investigated for use as vaccine antigens to control these parasites. Sea lice, such as *L. salmonis*, like other arthropod ectoparasites, produce biologically active substances at the site of attachment and feeding that limits the host immune response. As these substances have potential for use in a vaccine against sea lice we have identified a number of these substances from *L. salmonis* and have examined their effects of host immune function *in vitro*.

Potential antigens have been identified using a combination of molecular biological, proteomic, biochemical and immunological techniques. For example, an increase in protease activity has been observed in the mucus of *L. salmonis* infected Atlantic salmon, compared to non-infected fish (Ross, N.W., *et al.*, Dis Aquat Org 41: 43-51, 2000; Fast, M.D., *et al.*, Dis Aquat Org 52: 57-68, 2002). This increased activity is primarily due to the appearance of a series of low molecular weight (18-24 kDa) proteins, that are produced by *L. salmonis* and were identified as trypsins based on activity, inhibition studies and size. Trypsin activity was identified in infected salmon mucus using aminobenzamidine affinity adsorption and protease zymography (Firth, K.J., *et al.*, J Parasitol 86: 1199-1205, 2000). Several genes encoding for trypsin have been characterized from *L. salmonis* and the site of trypsin expression determined (Johnson, S.C., *et al.*, Parasitol Res 88: 789-796, 2002; Kvamme, B.O., *et al.*, Int. J. Parasitol. 34, 823-832, 2004; Kvamme, B.O. *et al.,* Gene 352:63-72, 2005).

Several cDNA libraries have been developed from the copepodid, preadult and adult stages of *L. salmonis*. An expressed sequence tag (EST) study of the preadult library resulted in the identification of a number of genes encoding trypsin and related proteases (including chymotrypsin and others in the peptidase S1 family), heat shock proteins, cuticle proteins and metabolic enzymes. Some of these genes as described herein have utility as antigens in a sea lice vaccine.

Trypsin-like activity is secreted by *L. salmonis* onto the salmon skin and is believed to be used by the sea lice to feed on the salmon mucus, skin and blood and to protect the sea lice from the salmon immune response (Firth, *et al. supra*). Trypsin was discovered in the secretion products (SPs) of sea lice, following stimulation with dopamine, by amino acid sequencing using mass-spectrometry. Table 1 shows the peptide sequences of *L. salmonis* secreted trypsin. Protection against sea lice trypsin may reduce the feeding of the lice and reduce the suppression of the immune response.

**Table 1:** Summary of *L. salmonis* secreted trypsin identified from LC/MS/MS

| Sea Lice protein matches | Assoc. Fraction (pool#-fraction#) | Parent Ion (m/z) | Mr (Da) | Error (ppm) [a] | Score [b] | Peptide sequence (Start-end) [c] |
|---|---|---|---|---|---|---|
| Sea Lice Trypsin (types 1-4) | 1-2 | 579.80 | 1157.77 | 27 | 46 | [215]FIDWIAEHQ[223] (SEQ ID NO: 25) |
| | 1-1 | 638.35 | 1274.69 | 38 | 72 | [71]IAVSDITYHEK[81] (SEQ ID NO: 26) |
| | 3-6 | 920.18 | 1840.28 | 13 | 25 | [115]DQEFIGDVVVSGWGTISSSGPPSPVLK[141] (SEQ ID NO: 27) |
| *SL-0903* vitellogenin-like | 1-1 | 580.28 | 1158.48 | 46 | 27 | NQYDEFESK (SEQ ID NO: 28) |
| *SL-1469* *SEP protein 3* | 1-1 | 724.85 | 1447.66 | 17 | 24 | LSFEHETTEEAR (SEQ ID NO: 29) |
| | 1-2 | 879.98 | 1757.91 | 29 | 72 | IILGHEFTPGYIENR (SEQ ID NO: 30) |
| *SL-0547* *SEP protein 1* | 1-1 | 604.31 | 1204.67 | 19 | 25 | IVILKELSSGM (SEQ ID NO: 31) + M oxidation[d] |
| *SL-0858* *SEP Protein 2* | 1-2 | 1248.71 | 2495.33 | 65 | 35 | AGQYGGEISGIVLPNIPPSISNLAK (SEQ ID NO: 32) |

*a* Difference (in parts-per-million) between measured mass and mass predicted from the DNA sequence.

*b* Score from MASCOT™ search, scores above 21 indicate identity or extensive homology ($p<0.05$)

*c* Cyanogen bromide/tryptic peptide sequence predicted from the DNA sequence.

*d* +M oxidation means that the MASCOT match was for a peptide containing an oxidized methionine residue.

Vitellogenin-like protein was discovered in the secretion products (SPs) of sea lice following stimulation with dopamine. Vitellogenin has previously been reported as an effective antigen in a tick vaccine (Tellam, R.I., *et al.*, Vet Parasitol 103: 141-156, 2002). Inclusion of vitellogenin in a sea lice vaccine may interfere with the fecundity of sea lice and reduce the number of offspring and hence reduce future numbers of sea lice. In addition, vitellogenin-like proteins have been implicated in the synthesis of melanin in invertebrates (Lee, K.M. *et al.,* Eur J Biochem 267:3695-3703, 2000). Melanin is an important defence molecule of invertebrates.

Mussel adhesion-like genes express proteins similar to those found in the mussel byssus threads that mussels use to attach themselves to solid surfaces. How these genes relate to sea lice infestation is not currently understood, but they may be involved in the production of frontal filaments. The frontal filament is used by chalimus stages to physically attach themselves to the host (Gonzalez-Alanis, P., *et al.*, J Parasitol 87: 561-574, 2001).

BCS-1 genes are expressed by barnacles when they switch from a planktonic form to an attached form (Okazaki,Y., *et al.*, Gene 250 (1-2): 127-135, 2000). There is currently evidence to suggest that these are cuticle-binding proteins. Disruption of these proteins by antibodies may interfere with moulting, integrity of the sea lice cuticle and normal growth of the lice.

Secretory proteins produced by the sea lice may act as immunomodulatory agents or assist in the feeding activities on the host (Fast, M.D., *et al.*, Exp Parasitol. 107:5-13, 2004; Fast, M.D., *et al.,* J Parasitol 89: 7-13, 2003). Neutralization of these activities by host-derived antibodies may impair sea lice growth and survival on salmon.

Vaccines are generally safer than chemical treatments, both to the fish and to the environment. However, no commercial vaccines against sea lice have been developed to date. Vaccine development has been hindered by a lack of knowledge of the host-pathogen interactions between sea lice and their hosts. There appears to be very limited antibody response in naturally infected hosts. Experimental vaccines, particularly through whole-animal extracts, have been produced against *L. salmonis*. Investigations in the development of sea lice vaccines have targeted immunogenic proteins from sea lice and, in particular, targeting gut antigens. These vaccines, based on whole animal extracts, have not been shown to be protective though their administration did result in minor changes in *L. salmonis* fecundity (Grayson T.H., *et al.,* J Fish Biol 47: 85-94, 1995). This particular study, however, was a one-time trial and no further results have been reported from this group. Liposome-based fish vaccines in certain species of fin-fish have also been explored (Keough, PCT Application WO 03/101482) but not in combination with sea lice antigens.

A more recent discussion of possible vaccine targets in the gut was put forth by Raynard *et al.*; however, their studies have been met with limited success (Raynard, R.S., *et al.*, Pest Manag Sci 58: 569-575, 2002).

**Promiscuous T-cell epitopes**

Promiscuous T-cell epitopes (or "PTC epitopes") are highly immunogenic peptides that can be characterized in part by their capacity to bind several isotypic and allotypic forms of human MHC class II molecules. By helping to bypass MHC restriction, they can induce T-cell and antibody responses in members of a genetically diverse population expressing diverse MHC haplotypes. The PTC epitopes can therefore be combined with antigens that, by themselves, are poorly immunogenic, to generate potent peptide immunogens. In the present invention, these epitopes are incorporated into the composition to enhance the immunogenicity of the antigen, and the composition overall, in a broad range of species.

Promiscuous T-cell epitopes can be derived from naturally occurring immunogens of viral and bacterial origin. Naturally occurring PTC epitopes can also be conservatively modified by single- or multiple-amino acid additions, deletions or substitutions (e.g. within classes of charged, hydrophilic/hydrophobic, steric amino acids) to obtain candidate sequences that can be screened for their ability to enhance immunogenicity.

Non-naturally occurring PTC epitopes can be artificially synthesized to obtain sequences that have comparable or better immunogenicity. Artificial PTC epitopes can range in size from about 15 to about 50 amino acid residues in length and can have structural features such as amphipathic helices, which are alpha-helical structures with hydrophobic amino acid residues dominating one face of the helix and charged or polar residues dominating the surrounding faces. The PTC epitopes may also contain additional primary amino acid patterns, such as a Gly or a charged residue followed by two to three hydrophobic residues, followed in turn by a charged or polar residue (a Rothbard sequence). In addition, PTC epitopes often obey the 1, 4, 5, 8 rule, where a positively charged residue is followed by hydrophobic residues at the fourth, fifth, and eighth positions after the charged residue.

These features may be incorporated into the designs of artificial PTC epitopes. Variable positions and preferred amino acids are available for MHC-binding motifs (Meister et al., Vaccine, 1995; 13:581-591). For example, the degenerate PTC epitope described in WO 95/11998 as SSAL1TH1 has the degenerate sequence (Asp/Glu)-(Leu/Ile/Val/Phe)-Ser-(Asp/Gly)-(Leu/Ile/Val/Phe)-(Lys/Arg)-Gly-(Leu/Ile/Val/Phe)- (Leu/Ile/Val/Phe)-(Leu/Ile/Val/Phe)-His-(Lys/Arg)-Leu/Ile/Val/Phe)-(Asp/Glu)-Gly-(Leu/Ile/Val/Phe)-.

**Specific examples of PTC epitopes**

Particularly useful promiscuous T-cell epitopes are measles virus protein F LSEIKGVIVHRLEGV (SEQ ID NO: 33); or tetanus sequence QYIKANSKFIGITEL (SEQ ID NO: 34).

Examples of particularly useful promiscuous T-cell epitopes are listed in Table 2:

**Table 2:** Examples of Promiscuous T-cell Epitopes

| ***description*** | ***amino acid sequence*** | ***SEQ ID NO:*** |
|---|---|---|
| measles 289-302 | LSEIKGVIVHRLEGV | 33 |
| tetanus toxin 830- 844 | QYIKANSKFIGITEL | 34 |

Because of a lack of understanding of the mechanisms and pathology surrounding sea lice infestation of salmon, identification of suitable targets to treat the disease has not been successful. This has hampered the progress of vaccine research and as such, despite the promise and success of vaccine-based therapies in other areas of infection, a suitable sea lice vaccine has yet to be developed. Consequently, there is a need to provide effective suitable molecular targets (antigens) and a vaccine against sea lice infection.

## SUMMARY OF THE INVENTION

It is an object of the present invention to obviate or mitigate at least one disadvantage of previous treatments against sea lice infection in fish.

In a first aspect, the present invention provides a vaccine against caligid copepod infection in fish, the vaccine comprising an immunologically effective amount of antigen. Particularly, the caligid copepod is *Lepeophtheirus salmonis*, although any copepod infection may be treated. In one embodiment, the vaccine comprises a nucleotide or peptide fragment of *L. salmonis* trypsin and a pharmaceutically-acceptable adjuvant, diluent or carrier.

In another aspect of the present invention there are provided DNA and amino acid sequences encoding antigens for use in the preparation of vaccine formulations for the treatment of caligid copepod infection in fish. Embodiments of these sequences include secretory products (SEPs) 1, 2 and 3, vitellogenin-like protein, melanization-related protein, adhesion protein 1 and 2, and cuticle binding protein 1.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

## BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will now be described, by way of example only, with reference to the attached Figures, wherein:

Fig. 1 shows the a) nucleic acid sequence and b) amino acid sequence of vitellogenin-like protein (SEQ ID NO: 1, 2).

Fig. 2 shows the a) nucleic acid sequence and b) amino acid sequence of SEP protein 1 (SEQ ID NO: 3, 4).

Fig. 3 shows the a) nucleic acid sequence and b) amino acid sequence of SEP protein 2 (SEQ ID NO: 5, 6).

Fig. 4 shows the a) nucleic acid sequence and b) amino acid sequence of SEP protein 3 (SEQ ID NO: 7, 8).

Fig. 5 shows the a) nucleic acid sequence and b) amino acid sequence of adhesion protein 2, homologous to mussel adhesive plaque matrix protein 2 precursor (SEQ ID NO: 9, 10).

Fig. 6 shows the a) nucleic acid sequence and b) amino acid sequence of adhesion protein 1, homologous to mussel adhesive plaque matrix protein precursor (foot protein 1) (SEQ ID NO: 11, 12)

Fig. 7 shows the a) nucleic acid sequence and b) amino acid sequence of cuticle binding protein 1, homologous to BSC-1 like protein (moran 9-15) (SEQ ID NO: 13, 14).

Fig. 8 is a graph of lice per cm of fish over duration of time post-infection, using vaccines (A/B = expressed sea lice trypsin gene with T-cell epitope; Y/Z = expressed sea lice trypsin gene; C = control) of the present invention.

Fig. 9 is a graph of lice per gram of fish over duration of time post-infection, using vaccines (A/B = expressed sea lice trypsin gene with T-cell epitope; Y/Z = expressed sea lice trypsin gene; C = control) of the present invention.

Fig. 10 shows a stacked data graph showing the percentages of different stages of sea lice present in fish immunized with *L. salmonis* trypsin vaccine as compared to control fish at each sampling time.

Fig. 11 shows a partial nucleic acid sequence (SEQ ID NO: 15) of vitellogenin-like protein SL-903 similar to but longer than the one in Fig. 1. Bolded, underlined and italicized ***TGA***, ***TAA*** or ***TAG*** are predicted stop codons.

Fig. 12 shows the full-length nucleic acid sequence of SEP protein 1 SL-0547 (SEQ ID NO: 16). Bolded and underlined **ATG** are presumed starting codons. Bolded, underlined and italicized ***TGA***, ***TAA*** or ***TAG*** are predicted stop codons.

Fig. 13 shows the putative full-length nucleic acid sequence of SEP protein 2 SL-0858 (SEQ ID NO: 17). Bolded and underlined **ATG** are presumed starting codons for protein. Bolded, underlined and italicized ***TGA***, ***TAA*** or ***TAG*** are predicted stop codons.

Fig. 14 shows the putative full-length sequence of SEP protein 3 SL-1469 (SEQ ID NO: 18). Bolded and underlined **ATG** are presumed starting codons for protein. Bolded, underlined and italicized ***TGA***, ***TAA*** or ***TAG*** are predicted stop codons.

Fig. 15 shows the putative full-length sequence of mussel adhesive protein SL-0927 (SEQ ID NO: 19). Bolded and underlined **ATG** are presumed starting codons for protein. Bolded, underlined and italicized ***TGA***, ***TAA*** or ***TAG*** are predicted stop codons.

Fig. 16 shows a partial amino acid sequence of vitellogenin-like protein SL-903 (SEQ ID NO: 20), together with BLAST ™ hits of the sequence. Underlined amino acids are the peptide fragments from Proteomics Mass Spectrometry data.

Fig. 17 shows the putative full-length amino acid sequence of SEP protein 1 SL-0547 (SEQ ID NO: 21), together with BLAST hits of the sequence. Underlined amino acids are the peptide fragments from Proteomics Mass Spectrometry data.

Fig. 18 shows the putative full-length amino acid sequence of SEP protein 2 SL-0858 (SEQ ID NO: 22), together with BLAST hits of the sequence. Underlined amino acids are the peptide fragments from Proteomics Mass Spectrometry data.

Fig. 19 shows the putative full-length amino acid sequence of SEP protein 3 SL-1469 (SEQ ID NO: 23), together with BLAST hits of the sequence. Underlined amino acids are the peptide fragments from Proteomics Mass Spectrometry data.

Fig. 20 shows the putative full-length amino acid sequence of mussel adhesive protein SL-0927 (SEQ ID NO: 24), together with BLAST hits of the sequence. Underlined amino acids are the peptide fragments from Proteomics Mass Spectrometry data.

Figure 21 shows mean (±SEM) expression of interleukin-1$\beta$ gene, relative to $\beta$-actin, in SHK-1 cells incubated with and without lipopolysaccharide (LPS), pooled *L. salmonis* secretory/excretory product fraction 1, pooled *L. salmonis* secretory/excretory product fraction 2, and pooled *L. salmonis* secretory/excretory product fraction 3. * indicates significant differences from control; † indicates significant differences from LPS + control.

Figure 22 shows mean (±SEM) expression of interleukin-1$\beta$ gene, relative to $\beta$-actin, in SHK-1 cells incubated with and without lipopolysaccharide (LPS), LPS and lyophilized liquid chromatography solvent (LC), *L. salmonis* secretory/excretory product fraction 1, *L. salmonis* secretory/excretory product fraction 2, and *L. salmonis* unfractionated secretory/excretory products. * indicates significant differences from control; † indicates significant differences from LPS + control.

## DETAILED DESCRIPTION

Generally, the present invention provides a vaccine for treating sea lice infection in fish, particularly infection from *L. salmonis*. It also relates to the DNA and amino acid sequence of molecular targets for use in the preparation of these vaccines.

The vaccines of the present invention were generated based on studies performed by our group and others on gene expression in sea lice. Several genes in sea lice that have the potential as producers of antigens in vaccine formulations designed to protect salmon against sea lice, especially *L. salmonis*. The genes include: 1) a gene for sea lice trypsin; 2) a gene having high similarity to a vitellogenin-like protein that is found in secretory products; 3) a gene having high sequence similarity to the mussel adhesion protein-1 gene; 4) a gene having high sequence similarity to the mussel adhesion protein-2 gene; 5) a number of genes having high sequence similarity to the gene coding for *Balanus amphrite* stage specific protein BCS-1; and, 6) genes coding for three secretory products (SP) proteins in sea lice that, at present, have no significant similarity with known proteins in public databases.

As used herein, an "antigen" refers to a molecule containing one or more epitopes that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is also used herein interchangeably with "immunogen."

As used herein, the term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used herein interchangeably with "antigenic determinant" or "antigenicdeterminant site."

Figures 1 through 6 show the sequences of the genes of the present invention described above and sequenced in our laboratory, with the exception of trypsin, of which the nucleic acid sequence has been published (Johnson, 2002, *supra)*. We have identified trypsin gene product in the sea lice secretions by amino acid sequencing using mass spectrometry. These genes were selected and investigated based on a prior understanding of their putative function.

Figures 11 through 20 show longer or putative full-length nucleotide and amino acid sequences of the genes and proteins as described herein.

Antigens derived from *L. salmonis* should provide protection for fish against other sea lice species as they are likely to use highly conserved methods to attach themselves to enable them to successfully feed on the host.

Adjuvants which can be used in the context of the present invention include Montanide™ ISA and IMS Adjuvants (Seppic, Paris, France), other oil-in-water, water-in-oil, and water-in-oil-in-water adjuvants, Ribi's Adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT), Hunter's TiterMax (CytRx Corp., Norcross, GA), aluminum salt adjuvants, nitrocellulose-adsorbed proteins, encapsulated antigens, nanoparticle containing adjuvants. Preferred adjuvants include Seppic Montanide 720, Montanide IMS111x, Montanide IMS131x, Montanide IMS221x, Montanide IMS301x, Montanide ISA206, Montanide ISA 207, Montanide ISA25, Montanide ISA27, Montanide ISA28, Montanide ISA35, Montanide ISA50A, Montanide ISA563, Montanide ISA70, Montanide ISA51, Montanide ISA720, Montanide ISA264. Particularly preferred adjuvants include, Montanide ISA740, Montanide ISA773, Montanide ISA 708, Montanide ISA266. The recommended adjuvant is Montanide ISA763.

Data from studies using the vaccines of the present invention for the treatment of sea lice infection are provided herein by way of the following examples.

**EXAMPLE 1**

Salmon were challenged with *L. salmonis* trypsin as the antigen. The fish were immunized with two formulation groups of trypsin vaccine: A/B (recombinant sea lice trypsin with a T-cell epitope) and Y/Z (recombinant sea lice trypsin only). Certain fish were administered with a control vaccine, C, containing adjuvant only. Protection of the fish is apparent at days 6, 11 and 20 (Figures 8 and 9). The number of sea lice per cm and per gram of fish is reduced in the vaccinated fish as compared to controls. The A/B vaccine formulation resulted in lower lice numbers than the Y/Z formulation showing that the inclusion of T-cell epitopes with sea lice antigens provide further protection against sea lice.

Figure 10 shows stacked data results of the challenge and vaccination experiments. The A/B (recombinant sea lice trypsin with a T-cell epitope) vaccine formulation appeared to slow the development of *L. salmonis,* as at days 6, 11 and 20, there were lower percentages of lice that had moulted to a more advanced stage compared to control fish

**EXAMPLE 2**

**Size Exclusion Chromatography and Protein Determination**

Lyophilized secretory excretory products (SEPs) were reconstituted with 1.0 M ammonium acetate (AMA) (pH 6.0). An Agilent 1100 HPLC equipped with a diode array detector (monitoring at 230 and 256 nm) and a Taso Haas (G3000PWX2, 6 µm $d_p$ (7.8 mm x 300 mm)) column were used to separate proteins/peptides in the secretions. These samples were then fractionated using a Waters Fraction collector according to time intervals. The fractions as shown in Table 1 were collected for 6 separate HPLC runs and pooled for each time interval. These samples were then freeze dried (-80°C) prior to protein determination. The column was kept at room temperature and eluted isocratically with 98:2 AMA: acetonitrile (ACN) for 30 minutes at 0.2 ml $min^{-1}$. Standard solutions of bovine serum albumin (BSA) (20 µg, 2.0 µg, and 0.2 µg), SW + DA, and bovine trypsin (40 µg) were all run as controls for peak comparison with SEPs.

Protein concentrations of *L. salmonis* secretory fractions were determined using a dye binding method (Bradford, M.M. Anal Biochem 72: 248-254, 1976). All assays were run on a Thermomax™ Microplate Reader (Molecular Devices). Samples were reconstituted in $ddH_2O$ and then, following protein determination, were split equally between cell-based functional assays and proteomic analysis.

**SHK Cell Culture**

SHK-1 cells were cultured at 18°C in 75 $cm^2$ tissue-culture-treated flasks (Costar), in L-15 medium (with 300 mg/L L-glutamine) supplemented with 500 $\mu$l gentamicin sulphate (50 mg/mL distilled in water), 365 $\mu$l 2-mercaptoethanol (55 mM in D-PBS) and 5% fetal bovine serum (FBS), as described by Fast *et al.* 2004 *supra*,. All media components were purchased from Gibco. Confluent flasks were passaged weekly by dividing cells and medium evenly between two flasks and adding an equal volume of new media to each flask. Cells used in this study were passaged between 64 and 68 times.

SHK-1 cells were seeded at approximately 4 x $10^6$ cells/flask in L-15 medium supplemented as described above. Cell stimulation followed the same procedure as in Fast, M.D. *et al.* Dev. Comp. Immunol. 29: 951-963, 2005. Briefly, following a 48 h period, to allow any manipulation-induced gene expression to return to constitutive levels, media was removed and 20 ml fresh media was added. Lipopolysaccharide (LPS) was added to all flasks, except the controls, to obtain a final concentration of 5 $\mu$g/mL.

In the first trial, SEP fractions were pooled into 3 groups (Table 1), each containing equal time ranges (10 min) and volumes from the size exclusion chromatography. This resulted in 13 $\mu$g protein (pooled fraction 1), 8.0 $\mu$g protein (pooled fraction 2) and <0.1 $\mu$g protein (pooled fraction 3) being added to each flask. These incubations were carried out for 4 h at 18°C before media was removed and cells stored in RNA later at –80°C until RNA extraction. This trial was repeated twice with triplicate flasks for each condition.

In the second trial, SEP fractions 1 and 2 from pooled fraction 1 (Table 1) were added at 1.0 and 1.4 $\mu$g per flask, respectively. These concentrations were attained after concentrating 4 size exclusion runs for each fraction. To test any affect of residual solvent on the cell-based assay, 4 blank runs of AMA underwent the same treatment and were included in the experiment as controls. Finally, the non-fractionated SEPs used in the macrophage incubations were incubated here at the same concentration (660 $\eta$g).

These incubations were carried out in triplicate and followed the same procedure as the first trial.

**Real-Time PCR on Atlantic salmon genes**

Total RNA was isolated from SHK-1 cells stored in RNAlater™ with the Nucleospin™ RNA II kit (Clontech) and concentration measured by spectrophotometer. RNA samples underwent PCR to verify the lack of DNA contamination. Sequences for Real-time PCR primers were designed, tested and products sequenced as previously described by Fast *et al., supra* (2004; 2005). Real-time quantitative PCR was performed using an iCycler iQ™ Real-Time detection system and SYBR green kits (Bio-Rad) also previously described by Fast *et al., supra* (2004; 2005). To ensure no genomic DNA contamination added to the quantified cDNA, non-RT controls for each RNA isolation were run under PCR and observed by 2.5% agarose gel electrophoresis.

The PCR profile was as follows: an initial 3 min denaturation step at 95°C, followed by 40 cycles of denaturation (30 s at 95°C), annealing (30 s at 58°C) and extension (30 s at 72°C), and finishing with a final extension step of 72°C for 5 min. The sensitivity of reactions and amplification of contaminant products such as primer dimers, indiscriminately detected by SYBR green (ie. SYBR green binds to all double stranded DNA), were evaluated by amplifying 10 fold dilutions of the clones ($10^{-2}$ to $10^{-8}$ ng) and duplicate samples as well as by performing a blank without cDNA with each run. The relationship between the threshold cycle (Ct) and the log (RNA) was linear ($-3.5<\text{slope}<-3.2$) for all reactions. Copy numbers were estimated based on the molecular weight of clones and OD 260.

**Immunomodulatory activity of SEP proteins**

The SEPs were fractionated based on size and fractions were collected. In the first trial (Fig. 21), pooled fractions (PF1, PF2, PF3) were incubated with SHK-1 cells (a salmon macrophage-like cell line) in combination with lipopolysaccharide (LPS) and the expression of the interleukin-1$\beta$ gene was monitored in order to determine the immunomodulatory effect of the fractionated SEP proteins on immune gene expression. Interleukin-1$\beta$ gene was reduced in expression by all three pooled fractions in comparison to cells stimulated with LPS alone (Fig. 21). When individual fractions containing proteins were tested, interleukin-1$\beta$ gene expression was reduced by fraction 2. LC-MS analysis showed that Fraction 2 of pool 1 contained the SEP protein 1, SEP Protein 2 and trypsin. Evidence for immunomodulatory activity of SEP, which contains all described proteins, is presented in Fig. 22 where there is a significant decrease in LPS-induced expression of interleukin-1$\beta$ in the presence of total SEPs.

The above-described embodiments of the present invention are intended to be examples only. Alterations, modifications and variations may be effected to the particular embodiments by those of skill in the art without departing from the scope of the invention, which is defined solely by the claims appended hereto.

# DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS COMPREND PLUS D'UN TOME.**

**CECI EST LE TOME __1___ DE __2___**

NOTE: Pour les tomes additionels, veillez contacter le Bureau Canadien des Brevets.

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# JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE THAN ONE VOLUME.**

**THIS IS VOLUME __1___ OF __2___**

NOTE: For additional volumes please contact the Canadian Patent Office.

What is claimed is:

1. A vaccine against caligid copepod infection in fish, the vaccine comprising an immunologically effective amount of antigen and a pharmaceutically-acceptable adjuvant, diluent or carrier.

2. The vaccine of claim 1 wherein the caligid copepod is *Lepeophtheirus salmonis.*

3. The vaccine of claim 1 or 2 wherein the antigen is *L. salmonis* trypsin.

4. A DNA encoding vitellogenin-like protein.

5. A DNA encoding melanization-related protein.

6. A DNA encoding SEP protein 1.

7. A DNA encoding SEP protein 2.

8. A DNA encoding SEP protein 3.

9. A DNA encoding adhesion protein 2.

10. A DNA encoding adhesion protein 1.

11 A DNA encoding cuticle binding protein 1.

12. An amino acid sequence encoding vitellogenin-like protein.

13. An amino acid sequence encoding melanization-related protein.

14. An amino acid sequence encoding SEP protein 1.

15. An amino acid sequence encoding SEP protein 2.

16. An amino acid sequence encoding SEP protein 3.

17. An amino acid sequence encoding adhesion protein 2.

18. An amino acid sequence encoding adhesion protein 1.

19. An amino acid sequence encoding cuticle binding protein 1.

20. A DNA vaccine comprising a DNA according to any one of claims 4 to 11 and a pharmaceutically-acceptable adjuvant, diluent or carrier.

21. A peptide vaccine comprising an amino acid sequence according to any one of claims 12 to 19 and a pharmaceutically-acceptable adjuvant, diluent or carrier.

22. A peptide vaccine comprising a measles-derived T-cell epitope and a pharmaceutically-acceptable adjuvant, diluent or carrier.

# FIG. 1

a)

**Vitellogenin-like protein (SL-0903)**

Nucleic acid sequence

GCAATACAAAATTATCCATGGATGTACTACCACAATTGACCATGTATATACTCTT
GACAATGTCACATATCCTTACACACCTACCTCATGCTGGACGTTGGCTTCTGGA
CACTGTTCCCCACATCCAACTTATGCAGTTTTTGTCAAAAAGTCTGCAGGATCTC
ATTTAGATGCTAAAATTTATTTGGGCGGTCACAGCATCGAATTCCAAACAAGTGG
CCCAAAGAAGATTAATGTTCTCATCAACGGTGAAGCTATTGATGTAGGAGAGGA
GGAACATGTTCATGAACAAGACGGACAAGAAATTTTCAAGGTCTTAAAATGGGG
ATCAAGTTACAGTGTTTACTCCTTCTTGAAAATCTGGGTAGTCTATGATGGCCAT
GCAGTCAGCTTAATCCCTGCTCCATCTGTTACGGGTCAACATTGCGGTTTGTGT
GGAAACTTCAACAGAAACCAATATGATGAATTTGAAAGCAAGGATGCTCATCAAT
TGAAGACATCCGACGAGCTTGTTGAAGATTATAAATGGAAGTGTTGAGAATTTAT
TTCTTTCATCGATTGATGGAGATATTTTTTACAATGATTCTATTATAT

b)

Amino Acid sequence

Q Y K I I H G C T T T I D H V Y T L D N V T Y P Y T P T S C W T L A S G H C S P H P
T Y A V F V K K S A G S H L D A K I Y L G G H S I E F Q T S G P K K I N V L I N G E
A I D V G E E E H V H E Q D G Q E I F K V L K W G S S Y S V Y S F L K I W V V Y D
G H A V S L I P A P S V T G Q H C G L C G N F N R N Q Y D E F E S K D A H Q L K T
S D E L V E D Y K W K C

# FIG. 2

## a)

**SEP Protein 1 (SL-0547)**

Nucleic acid sequence

GTCCGGTTCGGCAAATCTAAAATGTACCACAAGAAGGCAATCTATAAATTCTTGA
AGAAGACAACTCCCAAAAAGGTTGAGGCCAGTAAGCCCGCCTTCGTTGAGAAG
AAGGTCGGAGGTGCCAAGAATGGGGGTACTCGTATGGTTCGCGTCAAGAAGTT
GAAGAACGACTTCCCCACCATGGAAAGACGTGCTCATAGAATCGCCAAGAAGC
CTGAAAAGCTCTCTCGCAGGGTCCGTCCTACCCTCACCCCTGGAACTATTGCAG
TTATTCTTGCAGGTATCCACAAAGGAAAGAGAATCGTCATTCTCAAGGAGCTCTC
CAGTGGAATGCTTCTGATTTCTGGCCCCTTCAAGCTTAATAACTGCCCAATTAGA
AGGATTAATCAACGCTATTTGTTGGCCACATCAACCAAGCTCGATGTTTCATCCA
TTAAAATGCCCGAGAACATTAATGATGATTACTTCCGTCGTTTAAGAGCCGCCAA
GAAGCCAGCTGGTANTGTAT

## b)

Amino Acid sequence

V R F G K S K **Met** Y H K K A I Y K F L K K T T P K K V E A S K P A F V E K K V G G
A K N G G T R **Met** V R V K K L K N D F P T **Met** E R R A H R I A K K P E K L S R R V
R P T L T P G T I A V I L A G I H K G K R I V I L K E L S S G **Met** L L I S G P F K L N N
C P I R R I N Q R Y L L A T S T K L D V S S I K **Met** P E N I N D D Y F R R L R A A K
K P A G X V

# FIG. 3

a)

**SEP Protein 2 (SL-0858)**

Nucleic acid sequence

AAGCCCTCTTTGATCTTCAGAAGGACCTCGATGGAGATGTGGATGTTGAACTCA
AGTTGGTCAAGAAAGGAATTGTCTCCATTCCCATCCCCTGCATTGAAAGCCCTT
CAGGCCTGCATTTGGGCTCTTGTTCCTACAAACTGGAGGAGATTGTCAGCAAAT
ATGCGTATTTCTTGTGTCCAGACTATTTTCCAGAGGGCCAAAGCTGTTCTTTCCC
TTTGAAGGCAGGACAATATGGAGGTGAGATCTCTGGTATTGTTCTACCTGACAT
CCCACCTTCCATTTCCAACCTCGCCAAGGGAACCATTCATGGAACTCTCTCTGT
GACCAGAAACGGGGAAGAAGTTTTCTGTATCAATGGAGATTTGAAAATGACAAA
TTAATCCTTGTTTCTGTATCCATATTTGAAGTCAATAAAATACTTCATTAATCT

b)

Amino Acid sequence

-A L F D L Q K D L D G D V D V E L K L V K K G I V S I P I P C I E S P S G L H L G S
C S Y K L E E I V S K Y A Y F L C P D Y F P E G Q S C S F P L K A G Q Y G G E I S G I
V L P D I P P S I S N L A K G T I H G T L S V T R N G E E V F C I N G D L K **Met** T N
**Stop**

# FIG. 4

## a)

**SEP protein 3 (SL-1469)**

Nucleic acid sequence

AGAAATTGAAATCTCACTAAGATTTAATACTTCTCCCAATGGTGAGCGTTTATATT
TCAGAGGTAGAAAATGGGCTCTTACTGGTATCGTCAAAGCCAAAGGAGAACCAC
AAGATAGGGTATACAAAATTATTTTGGGACATGAATTCACTCCTGGATACATTGA
GAATCGTCTCAAGTTTAGAATGCAAAGAGTTGCAGTCCCTGGGCTTTTGTCTGA
TTATTCTATTTGCTTTAATATGGAAAACAAGTACCCAGACTTTGGAGAAGAGTTTA
TGACTTATGACAAGAGCACTCAATTAAAAATGTCTGGAAATGCAAGACTTCAATA
TGGTGCTGCTGCAGACTGTGACTCTACTCCTGGAGAAATGAAATTAAGCTTCGA
ACATGAAACAACTGAAGAAGCAAGAGAGGCTATGAAACACACGTGGTACTATGA
AAAGTGTATGGAACAGAAGCAACATCCAGAATGGGCAAGCAGAGGTGACAGAC
TTCCATTCACAGAAGCCTGCCACATGACAACATGGGATGCAACTACTGCTCCGT
AAATACACATGGAAGATGAACTTTGTTNAGATGACTGATCGCATTGAATGCTATT
GTTTC

## b)

Amino Acid sequence

-E I E I S L R F N T S P N G E R L Y F R G R K W A L T G I V K A K G E P Q D R V Y K
I I L G H E F T P G Y I E N R L K F R **Met** Q R V A V P G L L S D Y S I C F N **Met** E N
K Y P D F G E E F **Met** T Y D K S T Q L K **Met** S G N A R L Q Y G A A A D C D S T P G
E **Met** K L S F E H E T T E E A R E A **Met** K H T W Y Y E K C **Met** E Q K Q H P E W A
S R G D R L P F T E A C H **Met** T T W D A T T A P stop

# FIG. 5

## a)

**Adhesion protein 2**
**~Mussel adhesive plaque matrix protein 2 precursor (foot protein 2)**
**(mb3a02.ckr)**

Nucleic acid sequence

TACATGTCTTGAACCTGTCTGTCCATCTAATTTGTGTAAAAATGGTGGAAAATGT
AAAGTTTATCGTGGAGTATGTTATTGCGATTGTAAAGGTACTGGTTTTGAAGGTT
CAAAATGTCATCAACCAACTTGCACACCTACAACATGTCCTAAAAATGCTGTTTG
TGAACTGGATTGGTCTAATAAAAAAACACGTCGTACATGTAAAAAAGGATTTGCT
GGTGCAAATTGTGCTGATAATGCTTGCTCCCAGTTTCCATGTGAAAATGGATCT
GAATGTGTTGTAAAATATGGATCTCAACCACAATGTGATTGTAAGCCTGGATTTT
TTGGCAATTTCTGCCAATCACATTTTTGTGAACATTTCAAATATTCAGCTGTAGGT
GGTAAATGTGAAATTATGGAAGACGAAAAAAACTTGGGAGTCTATAAACCTGTTT
GTAAATGTTTAGAAGGATATGAAGGTAAAATTTGCAGCGAAATTTCTTGCTCTGA
ATCATTCTGTCATTATCGAGGAAAATGTTCCGTTAGTAAAGATATAAGAAGCTGC
AAATGTGACAGAGGAATTCAAGGCGAGCGCTGTGAACATCCTAAACCTTGTGAA
AGTTGCGACAAAGCAGATTGTGTTAGAGAAAAACCTGGCAGTGGCAATTTTGTT
TGTGCAAAAAAATAGACATAATATAATTAAG

## b)

Partial Amino Acid sequence

-T C L E P V C P S N L C K N G G K C K V Y R G V C Y C D C K G T G F E G S K C H
Q P T C T P T T C P K N A V C E L D W S N K K T R R T C K K G F A G A N C A D N A
C S Q F P C E N G S E C V V K Y G S Q P Q C D C K P G F F G N F C Q S H F C E H F K
Y S A V G G K C E I M E D E K N L G V Y K P V C K C L E G Y E G K I C S E I S C S E
S F C H Y R G K C S V S K D I R S C K C D R G I Q G E R C E H P K P C E S C D K A D
C V R E K P G S G N F V C A K K

# FIG. 6

## a)

**Adhesion protein 1**
**~Mussel adhesive plaque matrix protein precursor (foot protein 1) (sl-0927-9)**

Nucleic acid sequence

CCCATCCAAAGTAGCCTCAAAAACAAAGTCATATACCCGCCCTCTTTACTCCAAA
AAGAACTTCTTACGCAAGAAACCCTCTGTATATTACAAAGTTAAGAAAAATCCCG
TCAAGTTAAGAAAAGTTAAGAAAATTCTGAGACCTGTGAGTTACTCAACTCCTCA
GACTCATCCGAGCAGCACGACTGTTTCAACCACAAGAGACTATCCAAGTAAATC
TCTTGAAAGTTTAACGCAAACTAAGAGTCCAGAAATAGTATCGGCCTTTACTCCG
GTCTCAGTCTCAAAAAAGTCTATTAAATCATTGAATGCTCGAAAATCCATTTATGC
AAGTCCATCTACCCCATCTTTTAGATATTCACCAACAACACCTTCTTCATATCAAA
GTCTTAAGCCATTTGAGCCCAAACCTATTCATAGATTCAGATCTAAGCCAGGCTA
CAAGTCTACCAGATCCAAACCTACTTATGTTTCGTCAACGACCACCCACCCAGC
GTATGTATCATCTACTATTAGTCCAGCCTATGCATCATCTAGTGTTAGTCCAGCC
TATGCATCATCTAGTGTTAGCCCAGCCTATGCATC

## b)

Amino Acid sequence

P S K V A S K T K S Y T R P L Y S K K N F L R K K P S V Y Y K V K K N P V K L R K
V K K I L R P V S Y S T P Q T H P S S T T V S T T R D Y P S K S L E S L T Q T K S P E
I V S A F T P V S V S K K S I K S L N A R K S I Y A S P S T P S F R Y S P T T P S S Y Q
S L K P F E P K P I H R F R S K P G Y K S T R S K P T Y V S S T T T H P A Y V S S T I
S P A Y A S S S V S P A Y A S S S V S P A Y A

# FIG. 7

a)

**Cuticle binding protein 1**
**~BCS-1 like protein (moran 9-15)**

Nucleic acid sequence

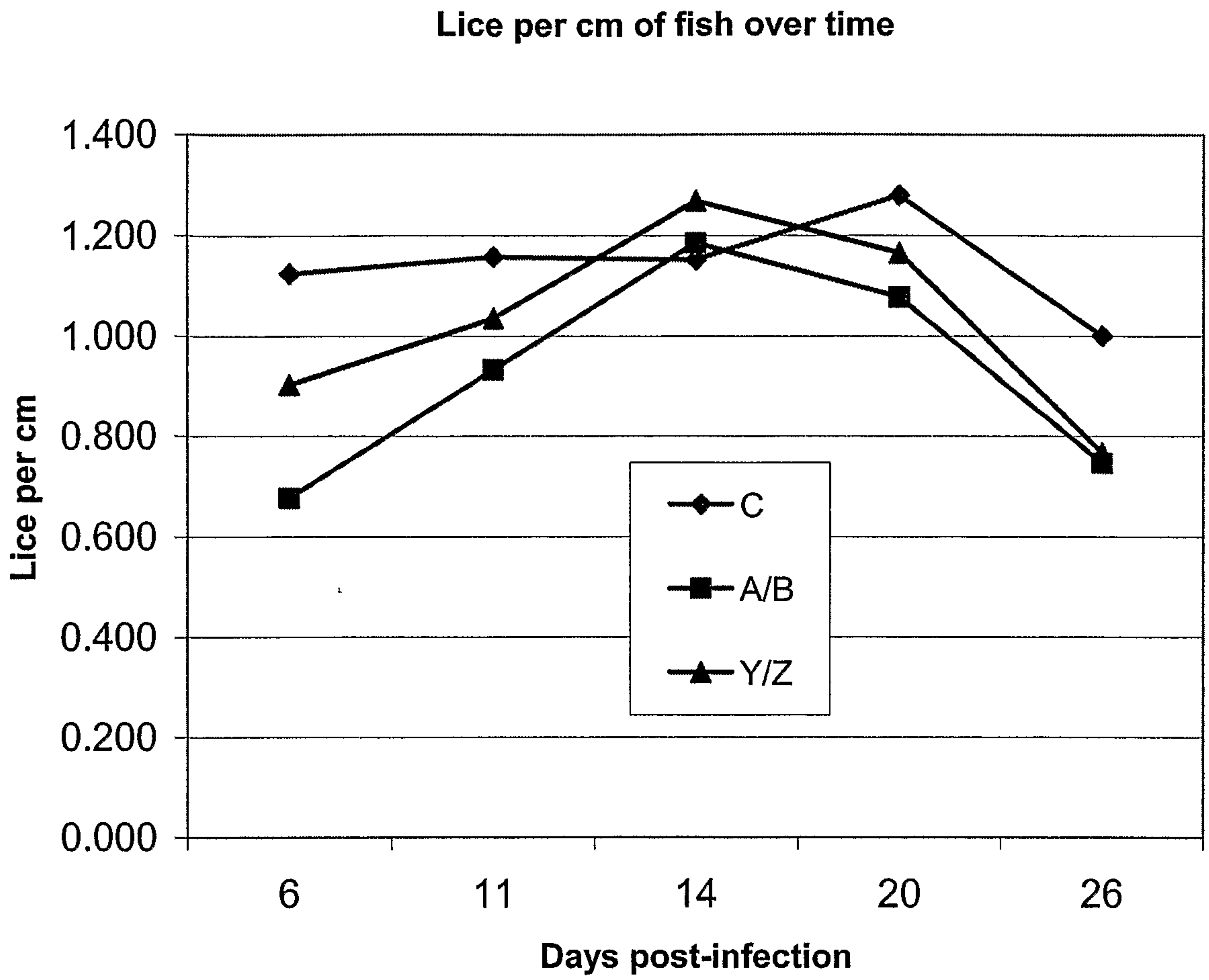
AGCGTGGTCGCGGCCGAGGTACGCGGGGAATCAGTACAAAGTTTACAGGCAAC
AATGATCATGTACACCTCAGTATTCCTTCTTTCCATCGTCTTTTCATGTGCCTTTG
GAGCACCCCAATATCAAGCCTCTCAGCCTGCTTATGCTCCAGTTGAAGAGCCTT
ACGCTTATCAATACGCCGTTCAAGATCCCCAATCAGGAAATGACTTTTCTGCCG
AAGAATCCTCTGATGGACAAGTCATCTCTGGATCCTATCAAGTGGCTCTCCCCG
ATGGTCGTATTCAAACTGTGACCTACACTGTGAGTGGAGATAGTGGATACGTTG
CTGATGTTCAGTATGAAGGAACTCCATCCTATCCCGAAGCTCCTCAGCAACCCA
GATATGTTTAAAAAGTTTGGCATTGATGAATATTCATTAATTGATTTATATTTATGT
TAAATATATTATTCATAGTTGAAATTGATATTGTTTACTCTCAAAGAAATACAAATT
TATATTTATATTTT

b)

Amino Acid sequence

S V V A A E V R G E S V Q S L Q A T M I M Y T S V F L L S I V F S C A F G A P Q Y Q
A S Q P A Y A P V E E P Y A Y Q Y A V Q D P Q S G N D F S A E E S S D G Q V I S G S
Y Q V A L P D G R I Q T V T Y T V S G D S G Y V A D V Q Y E G T P S Y P E A P Q Q
P R Y V

FIG. 8


Lice per cm of fish over time
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Lice per cm
6
11
14
20
26
Days post-infection
C
A/B
Y/Z

# FIG. 9

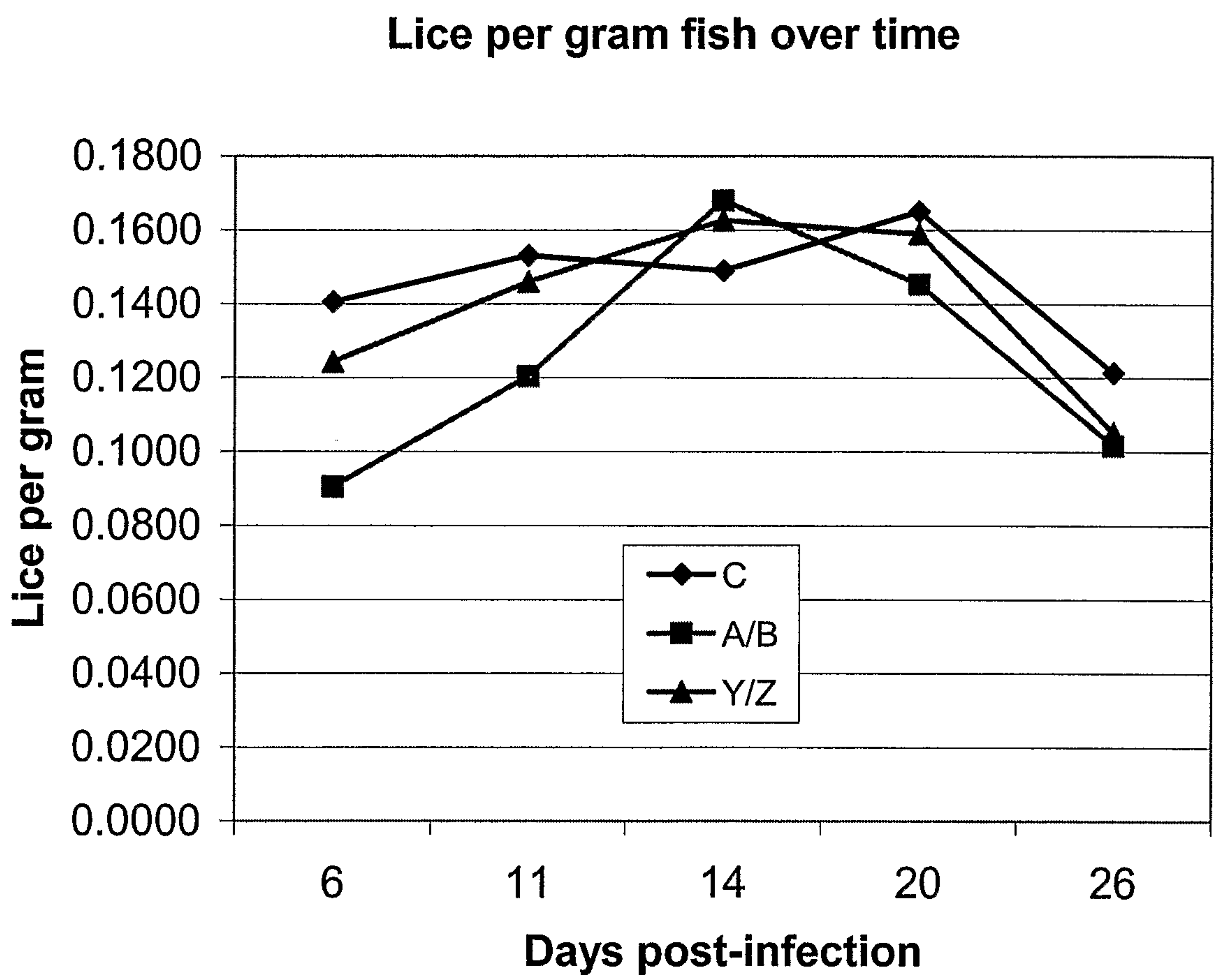
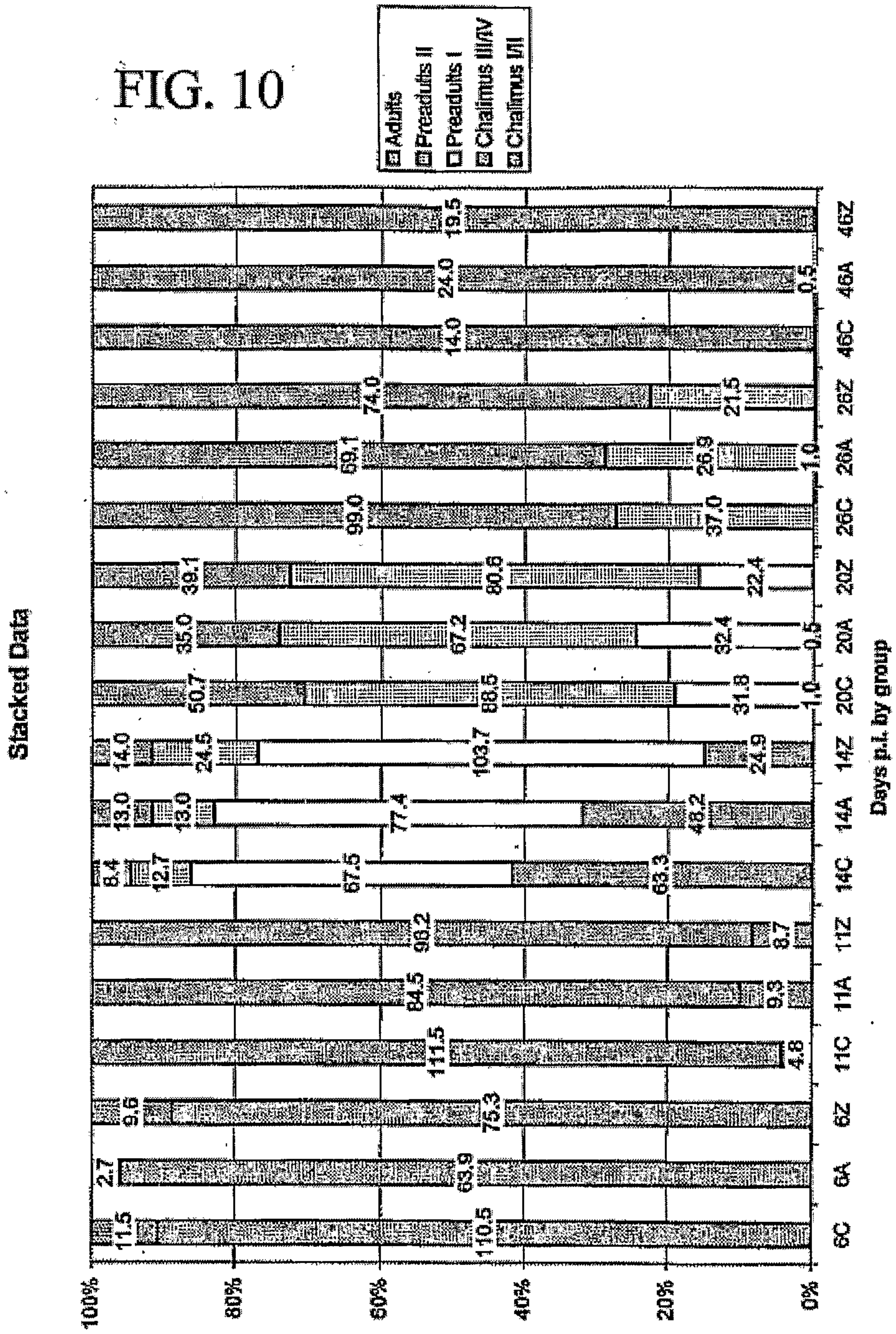

Lice per gram fish over time
Lice per gram
0.1800
0.1600
0.1400
0.1200
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
C
A/B
Y/Z
6
11
14
20
26
Days post-infection

FIG. 10


Stacked Data
Adults
Preadults II
Preadults I
Chalimus III/IV
Chalimus I/II
Days p.i. by group
100%
80%
60%
40%
20%
0%
6C
6A
6Z
11C
11A
11Z
14C
14A
14Z
20C
20A
20Z
26C
26A
26Z
46C
46A
46Z

# FIG. 11

**Vitellogenin-like protein SL-903** >SL0903FLG

TATTCCAACGTAACATGATCAGAGGATGGGCTCAAAGACTCCAATTGAACATGGATAAAATCAACAATCATGGAC
ATGGATTCCATTCTGAAGAGCAATCCATCTTTGGAGATTGTGATACTCTTTACACTGTTAGTGATCATAAAATTGTG
AAATCTGTAAGTCATACTAAAGATTGCAAAAACAGAGTACGTGTCCTCATTGATGATTGGAGGGGCGAACGCTGT
GACATTGACCCAGAGCATCCAGAAAGCAGAGAAAATCCAAATGGTCTTTACTCTGCTTCCAACACCATTTATGTCG
TGGACAAGAAGGGAGATCATTTCCACCCTAAGGCCATCATTGGATCATCCTCAGTTGTTGCACAATTCTATCAAAT
GGAAGGAGTCTCCTTTATTGCTCACTCTAATTCAACATCTATTTTGAAATCTGTTGAAGATATCTCAGAACCTATGG
TTGTTGTTGGAATCCCAGTCAAGGATCTCAAATACGAATTTGAAGATAAAGAATATCAATGGAATTCTGACAGAG
ACCTCAAGGCTCGTGAAGAACATTTATCCACTGGTGAATTTTTTGAGAGTGACATGTCCACTTTGTCAAAATATGT
TAAGGAAAAGCTCAACAAGTTCCATGACATCATGCAACATCTTTCAAATGACAAAGATGCCATTGCAGAAGCTCA
TGATAATGGAGTAAACAGTATGGTACCCGGTATGTTGGCTATGGACTACAATACATTGAAGGCTATGTCTGAAGA
ACTTCACTCAGACAAATCTGATGAAGGTGTATTCAAATACAATCTTTTCAATGAACTTTTGGGAAGTTTAGGAACC
AGCGCTTCTGCACTCCTGGTCCGTGACATGATCATGGAAGACAAATTTGAAAACTTCAGGGATGCTGTCCGTGCTC
TTACTGCCATTCCTTTCCATATTCGTCACCCTAACAAGCAACTTTTGAGTGAATTCGAAGCTTTGTACAACTACGAT
GGAGATCAACTCCTCAAAGATGCCGTCCCAATTGTTCTTGGACACTTAGCACGAGTTACCTGCGAAAGAGCCGGA
GTAATGCACTCCCCTGCTTCTAAAGAATGTTTCCACTCTGTTGTCGATGGATACGCCGATAAAACAATTGAAAAAA
TTATGGGTGCATCTGACCACAAAGAGCAAATTAAGCTTCTTGGAATGTTGTTCAATCTTCGCTATGGAAACGTTGC
TGAAAAATTAAAGCCTCTTATTTATGGAGAAACTGAGATCAAATGTGGACATCTCCGTACTCTTGCTGTTCAAGCT
GCTGCTTTTGGAGCAATCAACAACGGAAAGGGAGCTGAATATTTATTGCCCATTTTTGCGGACAGTGAAAACTCTC
ATGAACTTAGATTGACAGCTCTCTCATACTTGATGGATGCTCATCCCACCGCAACTCACTTTAACACCATCGTTGCT
GTACTTTACAGAGAAAAAGATTATGAAGTTATCAACTATGCTTTTACACTCTTGGATAAATATGCAACCAATATTA
ACCCCTGCAAAAAATCGGTCTCTGTATTGGCCAAATACTTCTTGAAATATCTTAAGCAATACAGTCATTTTGAAAC
TGACTATGGATTGGGAGTGTCCAAAACTTATAGTCGTCAATTCCAACAATCTAAATACGGGTATGGAGGTGAATAC
AGCTACTGGGTCATTGGATCTCATAGTTCTACCCTTCCTCTGAGTGTTGCAATGTGTATGGACACAACCTTGTTTGG
AGGATATACCGCTAATGGAATGTGTGTGCAATTAAGAATTGAAGGATTGTCCAAGGCTCTCATCCGTAAATTCAAG
ACGATGAGCCCAGATATCTGGAAATCTGAAGATCTCAAAAGTATCTTAATGGGAGATATGAACATTAAGGAAAGA
CCTGATCAACCAATCAACGTTGAGGTTCTCCTTTTCGTCAAGAACTCAGTTGTTGCATTCAGACAATATAACGAAG
ATTCAATTAAGGAAGGTGGGAACTTGAAAGAAATTTTCGATCGATTGAAAGGACTTGGAGACACCTACTCCATTA
ATCACCAAAGAGCAATGAGATTTGGAAGCCTCTTGTACCAACAACCCTTGGAAGTCGGTGCTCCAGTCTCTTACTT
AAACTCATTCACCGGCGTTTTTGATGTTCAAGCTACAATTAAGAAGGGAAATGCCAGAGGTCTTATGTTCAGAGAT
GTCAAATATAACATGAACTTTTTTGGACATGGATCTCGTATGATGATGGTTCAAAACCCACAATCAAAGATGTTTT
ATTCCATTTCTCAAAACCGCATTTATGGCTCCCATTTCCCAAGAGAATTTGTCATTGGAGTTAATCCATTGAAGAAA
GAGTTTAAATTATCTATTCAACGGCCTTCTTATGAGAATCCACTCGTACTTATGATGCACTCTTTAACAAAAGTTTA
CACTGGATCTCAAAATGTTAATGAAAAGCAGGATATTTCAGCCAACTGTCCTGAGTGCAAGTCTGATACTCCTGTA
TCGTATGGTCCAGATGCTGCTAAAACCAGAGTCTTTTTAAACCATGATTGCGATAAGACTGGCTCTTATATTCACG
GGGAATACTTTGACTGTGAAATGGAATCCAATAGGGGAAAGGTCTTATACCATTTGTGGAGAGCCATGTTACCTTA
TAACAAAAACCCTAAGACCTTTGGAAATGGTATTCGCATGGGTATTCGTCAAATCAGAGCTTATTTTGTTTTCTTCC
CAAGAGCTGAAAAATGTGGAGCAATGCTTCGCTGGTCACAATCAAAGGAAAATCCAGTTAAAGAGCTTGAAATTT
CTATGAGATTCAATGCTAATCCCAATGGCGAGCGTCTTTTCTTTAGAGGACGTAAATGGGTAGTCACAACTATTAT
CAAAGCCAAAGGAGAACCACAAGATAGGGTATACAAAATTATTTTGGGACATGAATTCACTCCTGGATACATTGA
GAATCGTCTCAAGTTTAGAATGCAAAGAGCTGCAGTTCCTGGAATTATGTCTGATTATTCTATTTGCTTCAATATGG
AAAACAAGTACCCAGACTTTGGAGAAGAGTTTATGACTTATGACAAGAGCACTCAATTAAAAATGACTGGAAAGG
CAAAACTTCAATATGGTGCTGCTGCAGACTGTGACTCTACTCCTGGAGAAATGAAATTAAGCTTCAAACATGAAAC
AACTGAAGAAGCAAGAGAGGCTATGAAACACACTTGGTACTATGAAAAGTGTATGGAACAGAAGAAACAGCCAG
AATGGGCAAACAGAGGTGATAAACTTCCATTCACACAAGCTTGCCACATGACAACATGGGATGCAACTACTGCAC
GTAAATACTCATGGAAGATGAACTTTGTTAAGATGACTGATCGCATGAATGCTATTGTTTCTCAATTCCAAAGTAT
CATGAAAACTGGTCTTTTACCTTACTGGGACATTGATCCAGAAATTATCCCAGCTACAAGTGCTGATCCCCACATG
AATATCAAAGCTACCCTTAAAAACCACGACAAGAATGTTGATATGTACATGGAAACCAGTCAGGGAGGTCAACGT
TTCAACGATATTCCTCTTAGTTTAAATTGGCGCCCAATGTTGAGAAACCTTAAGTTTACATCCACCACTAGACGTCT
CATGCAATACAAAATTATCCATGGATGTACTACCACAATTGACCATGTATATACTCTTGACAATGTCACATATCCT
TACACACCTACCTCATGCTGGACGTTGGCTTCTGGACACTGTTCCCCACATCCAACTTATGCAGTTTTTGTCAAAAA
GTCTGCAGGATCTCATTTAGATGCTAAAATTTATTTGGGCGGTCACAGCATCGAATTCCAAACAAGTGGCCCAAAG
AAGATTAATGTTCTCATCAACGGTGAAGCTATTGATGTAGGAGAGGAGGAACATGTTCATGAACAAGACGGACAA
GAAATTTTCAAGGTCTTAAAATGGGGATCAAGTTACAGTGTTTACTCCTTCTTGAAAATCTGGGTAGTCTATGATG
GCCATGCAGTCAGCTTAATCCCTGCTCCATCTGTTACGGGTCAACATTGCGGTTTGTGTGGAAACTTCAACAGAAA
CCAATATGATGAATTTGAAAGCAAGGATGCTCATCAATTGAAGACATCCGACGAGCTTGTTGAAGATTATAAATG
GAAGTGT***<u>TGA</u>***GAATTTATTTCTTTCATCG

# FIG. 12

**SEP protein 1 SL-0547**
>SL0547DRFL

CTTCTTAAATCCTGAAAAGGTAGTAGTAGAACTTCCACGGAA**ATG**GCGAA
GAACAAGAACGTCGGTAAGCCGAGGAACTACAAGTTAGCCTCCGGAGTC
GTCCGGTTCGGCAAATCTAAAATGTACCACAAGAAGGCAATCTATAAATT
CTTGAAGAAGACAACTCCCAAAAAGGTTGAGGCCAGTAAGCCCGCCTTCG
TTGAGAAGAAGGTCGGAGGTGCCAAGAATGGGGGTACTCGTATGGTTCGC
GTCAAGAAGTTGAAGAACGACTTCCCCACCATGGAAAGACGTGCTCATAG
AATCGCCAAGAAGCCTGAAAAGCTCTCTCGCAGGGTCCGTCCTACCCTCA
CCCCTGGAACTATTGCAGTTATTCTTGCAGGTATCCACAAAGGAAAGAGA
ATCGTCATTCTCAAGGAGCTCTCCAGTGGAATGCTTCTGATTTCTGGCCCC
TTCAAGCTTAATAACTGCCCAATTAGAAGGATTAATCAACGCTATTTGTTG
GCCACATCAACCAAGCTCGATGTTTCATCCATTAAAATGCCCGAGAACAT
TAATGATGATTACTTCCGTCGTTTAAGAGCCGCCAAGAAGCCAGCTGGTA
GTGTATTCGAAGGTAAAAAGGAAGAATACAAACCTTCTGAACAACGTAAG
AAGGACCAAGTCGAAGTTGATAAGCAGCTCCTCAATGTCATCATGAAGCA
CCCCGAAGCCTCTCTTTTGAAACAATACTTGAAGAAGTCCTTCGGTCTTAG
CAAGGGACAATATCCTCATAATATGAAATTT***TAA***TTGTCGTCTTGTTAAAT
AAAACTAAAATTCCTCGC

# FIG. 13

**SEP protein 2 SL-0858**
>SL0858C4

TCAATTTGATTGATCCAGGAAGAGAAAC**ATG**AAGATCATTGCCATCTTTG
CACTTTTGTTCATTGCCGTGTCTGGAGAGGATCTGGAGTGGGAGTCCTGCA
ATCCCGATAACTTGGGAGAAGGAGACATTGCCCTCTCTCCTTATCCCCTTC
CAGTAGTTAGTGGAACCAGTCTGGATTTGAAAGCCCTCTTTGATCTTCACA
AGGACCTCGATGGAGATGTGGATGTTGAACTCAAGTTGGTCAAGAAAGGA
ATTGTCTCCATTCCCATCCCCTGCATTGAAAGCCCTTCAGGCCTGCATTTG
GGCTCTTGTTCCTACAAACTGGAGGAGATTGTCAGCAAATATGCGTATTTC
TTGTGTCCAGACTATTTTCCAGAGGGCCAAAGCTGTTCTTTCCCTTTGAAG
GCAGGACAATATGGAGGTGAGATCTCTGGTATTGTTCTACCTGACATCCC
ACCTTCCATTTCCAACCTCGCCAAGGGAACCATTCATGGAACTCTCTCTGT
GACCAGAAACGGGGAAGAAGTTTTCTGTATCAATGGAGATTTGAAAATGA
CAAAT***TAA***TCCTTGTTTCTGTATCC
ATATTTGAA

# FIG. 14

**SEP protein 3 SL-1469**
>SL1469FLRD

CCATTCAAGATGAGAGTCACAGCCCTCCTTTGCTTATTTGTTGCCGCTGTCAGCGGAAGCATATTTGAAGATGGAA
AGCAATATGTATTTGATTCAGAGACATCTGTTGTTGTTGGTACGATGGACCATGCTCCACACTCATCTGGATTTGC
TTACAAGCACCATACTACCATGCAAGTTCAAGGAGATAACATCAAAGTGAAGCTTTCTGACGTTGAATTCTCACAA
TTCAATGGAAAACATGAGAATGGAGAGTTCCCATTTGACCACACTAACTTTGTTGCAACAAACAGAGACATTCCTG
CATTTGAAGTTCAGTTGGACTCCCATGGATTGTTTTCATCCCTCAAAGTTGGTCCTAAACTGACACTATTCCAACG
TAACATGATCAGAGGATGGGCTCAAAGACTCCAATTGAACATGGATAAAATCAACAATCATGGACATGGATTCCAT
TCTGAAGAGCAATCCATCTTTGGAGATTGTGATACTCTTTACACTGTTAGTGATCATAAAATTGTGAAATCTGTAA
GTCATACTAAAGATTGCAAAAACAGAGTACGTGTCCTCATTGATGATTGGAGGGGCGAACGCTGTGACATTGACCC
AGAGCATCCAGAAAGCAGAGAAAATCCAAATGGTCTTTACTCTGCTTCCAACACCATTTATGTCGTGGACAAGAAG
GGAGATCATTTCCACCCTAAGGCCATCATTGGATCATCCTCAGTTGTTGCACAATTCTATCAAATGGAAGGAGTCT
CCTTTATTGCTCACTCTAATTCAACATCTATTTTGAAATCTGTTGAAGATATCTCAGAACCTATGGTTGTTGTTGG
AATCCCAGTCAAGGATCTCAAATACGAATTTGAAGATAAAGAATATCAATGGAATTCTGACAGAGACCTCAAGGCT
CGTGAAGAACATTTATCCACTGGTGAATTTTTTGAGAGTGACATGTCCACTTTGTCAAAATATGTTAAGGAAAAGC
TCAACAAGTTCCATGACATCATGCAACATCTTTCAAATGACAAAGATGCCATTGCAGAAGCTCATGATAATGGAGT
AAACAGTATGGTACCCGGTATGTTGGCTATGGACTACAATACATTGAAGGCTATGTCTGAAGAACTTCACTCAGAC
AAATCTGATGAAGGTGTATTCAAATACAATCTTTTCAATGAACTTTTGGGAAGTTTAGGAACCAGCGCTTCTGCAC
TCCTGGTCCGTGACATGATCATGGAAGACAAATTTGAAAACTTCAGGGATGCTGTCCGTGCTCTTACTGCCATTCC
TTTCCATATTCGTCACCCTAGCAAGCAACTTTTGAGTGAATTCGAAGCTTTGTACAACTACGATGGAGATCAACTC
CTCAAAGATGCCGTCCCAATTGTTCTTGGACACTTAGCACGAGTTACCTGCGAAAGAGCCGGAGTAATGCACTCCC
CTGCTTCTAAAGAATGTTTCCACTCTGTTGTCGATGGATACGCCGATAAAACAATTGAAAAAATTATGGGTGCATC
TGACCACAAAGAGCAAATTAAGCTTCTTGGAATGTTGTTCAATCTTCGCTATGGAAACGTTGCTGAAAAATTAAAG
CCTCTTATTTATGGAGAAACTGAGATCAAATGTGGACATCTCCGTACTCTTGCTGTTCAAGCTGCTGCTTTTGGAG
CAATCAACAACGGAAAGGGAGCTGAATATTTATTGCCCATTTTTGCGGACAGTGAAAACTCTCATGAACTTAGATT
GACAGCTCTCTCATACTTGATGGATGCTCATCCCACCGCAACTCACTTTAACACCATCGTTGCTGTACTTTACAGA
GAAAAAGATTATGAAGTTATCAACTATGCTTTTACACTCTTGGATAAATATGCAACCAATATTAACCCCTGCAAGA
AATCGGTCTCTGTATTGGCCAAATACTTCTTGAAATATCTTAAGCAATACAGTCATTTTGAAACTGACTATGGATT
GGGAGTGTCCAAAACTTATAGTCGTCAATTCCAACAATCTAAATACGGGTATGGAGGTGAATACAGCTACTGGGTC
ATTGGATCTCATAGTTCTACCCTTCCTCTGAGTGTTGCAATGTGTATGGACACAACCTTGTTTGGAGGATATACCG
CTAATGGAATGTGTGTGCAATTAAGAATTGAAGGATTGTCCAAGGCTCTCATCCGTAAATTCAAGACGATGAGCCC
AGATATCTGGAAATCTGAAGATCTCAAAAGTATCTTAATGGGAGATATGAACATTAAGGAAAGACCTGATCAACCA
ATCAACGTTGAGGTTCTCCTTTTCGTCAAGAACTCAGTTGTTGCATTCAGACAATATAACGAAGATTCAATTAAGG
AAGGTGGAACTTGAAAGAAATTTTCGATCAATTGAAAGGACTTGGAGGCACCTACTCCATTAATCACCAAAGAGC
AATGAGATTTGGAAGCCTCTTGTACCAACAACCCTTGGAAGTCGGTGCTCCAGTCTCTTACTTAAACTCATTCACC
GGCGTTTTTGATGTTCAAGCTACAATTAAGAAGGGAAATGCCAGAGGTCTTATGTTCAGAGATGTCAAATATAACA
TGAACTTTTTTGGACATGGATCTCGTATGATGATGGTTCAAAACCCACAATCAAAGATGTTTTATTCCATTTCTCA
AAACCGCATTTATGGCTCCCATTTCCCAAGAGAATTTGTCATTGGAGTTAATCCATTGAAGAAAGAGTTTAAATTA
TCTATTCAACGGCCTTCTTATGAGAATCCACTCGTACTTATGATGCACTCTTTAACAAAAGTTTACACTGGATCTC
AAAATGTTAATGAAAAGCAGGATATTTCAGCCAACTGTCCTGAGTGCAAGTCTGATACTCCTGTATCGTATGGTCC
AGATGCTGCTAAAACCAGAGTCTTTTTAAACCATGATTGCGATAAGACTGGCTCTTATATTCACGGGGAATACTTT
GACTGTGAAATGGAATCCAATAGGGGAAAGGTCTTATACCATTTGTGGAGAGCCATGTTACCTTATAACAAAAACC
CTAAGACCTTTGGAAATGGTATTCGCATGGGTATTCGTCAAATCAGAGCTTATTTTGTTTTCTTCCCAAGAGCTGA
AAAATGTGGAGCAATGCTTCGCTGGTCACAATCAAAGGAAAATCCAGTTAAAGAGCTTGAAATTTCTATGAGATTC
AATGCTAATCCCAATGGCGAGCGTCTTTTCTTTAGAGGACGTAAATGGGTAGTCACAACTATTATCAAAGCCAAAG
GAGAACCACAAGATAGGGTATACAAAATTATTTTGGACATGAATTCACTCCTGGATACATTGAGAATCGTCTCAA
GTTTAGAATGCAAAGAGCTGCAGTTCCTGGAATTATGTCTGATTATTCTATTTGCTTCAATATGGAAAACAAGTAC
CCAGACTTTGGAGAAGAGTTTATGACTTATGACAAGAGCACTCAATTAAAAATGACTGGAAAGGCAAAACTTCAAT
ATGGTGCTGCTGCAGACTGTGACTCTACTCCTGGAGAAATGAAATTAAGCTTCAAACATGAAACAACTGAAGAAGC
AAGAGAGGCTATGAAACACACTTGGTACTATGAAAAGTGTATGGAACAGAAGAAACAGCCAGAATGGGCAAACAGA
GGTGATAAACTTCCATTCACACAAGCTTGCCACATGACAACATGGGATGCAACTACTGCTCCGTAAATAC

# FIG. 15

**(mussel adhesive protein) SL-0927**
>SL0927R61

CCAATTCTAGC*TAG*TCACTA*TGA*ACTAGTCAAAATAATTTATATACTGTTTTTATA
TTGATTTTTATTTATATATTATTACATCCT*TGA*GGACTTTTGAACAAAGGTGTTCGT
AGTAAACTTTTGAGTCATTATTACATAGTTGTCATACCTATTTCAAGGATAATAAG
GACGATCATC**ATG**AAAACATCTATTATTTTCTCACTGTATGTTCTGCCTTCTATAC
TGCACCTTGCTGTAAGTGAAGATAAAACTATTATAGCTGAAGACCTGACAGCTGT
AGAGTCACGCTATAAAGTAGATGCAAAACCCTCACCTTATGTTCCTCCACAACCA
GCACCAGATTTTGATTACTTTGCACCCACTGTATCTCCTTCTTTCTCCCCAATCGC
ATCTCCCTCTCCTTCTTCCCCTGTTACATCCTACTTTACACCCACAACCATTCCTCC
AGTGACTCCCTCGACCACAACGACCACTGTTACAACAACAACCCCTGCCACATCT
ACTTACAGAAAGTTATTCTTTCCAACGTCTTTCAAACCATCTTTTCTTTCTTCTCGA
AAAAAGTTAACCACAACTACAACAACGCCGGCTACAACATCCTCAACAACAACA
ACAATAACCTTCACCCCAACTACTTCACCCCCTCCATCTGCTAATGAAGTTCGAA
CAACATTAAACCCATCCAAAGTAGCCTCAAAAACAAAGTCATATACCCGCCCTCT
TTACTCCAAAAAGAACTTCTTACGCAAGAAACCCTCTGTATATTACAAAGTTAAG
AAAAATCCCGTCAAGTTAAGAAAAGTTAAGAAAATTCTGAGACCTGTGAGTTACT
CAACTCCTCAGACTCATCCGAGCAGCACGACTGTTTCAACCACAAGAGACTATCC
AAGTAAATCTCTTGAAAGTTTAACGCAAACTAAGAGTCCAGAAATAGTATCGGCC
TTTACTCCGGTCTCAGTCTCAAAAAAGTCTATTAAATCATTGAATGCTCGAAAAT
CCATTTATGCAAGTCCATCTACCCCATCTTTTAGATATTCACCAACAACACCTTCT
TCATATCAAAGTCTTAAGCCATTTGAGCCCAAACCTATTCATAGATTCAGATCTA
AGCCAGGCTACAAGTCTACCAGATCCAAACCTACTTATGTTTCGTCAACGACCAC
CCACCCAGCGTATGTATCATCTACTATTAGTCCAGCCTATGCATCATCTAGTGTTA
GTCCAGCCTATGCATCATCTAGTGTTAGCCCAGCCTATGCATCCACAACAGTTAA
ACCTGTCTTTGTTTCTACGACAGCAAATGAAATATACTATACGCCCGAACCAAAA
AGGGTACGAAGTCTTCCACTTACACGAGAACAAGCACATCTCTATTCATCTATTC
CTTATGATTCAACAACTACATCAAGGCAGCCTCCAGCTCCTGTTTCCTACAGTAC
ACCTAAGCCCCACTCGAAACAACATAGCCAATATCGCGAATTGCCATTGACTAGA
GAGCAAAGTGAAAACATTGAGTTTAGTACTCCCGTAAAGGCTAATACGAAGCCTT
ACAATAATAATATACAATTTAATCCTGTAAGACGGATTCCTTCTCATTATAGAAA
TCATCAAGCGTTGAATGAAATACGTCGGGAGGAAAAGTATCCTGCACAACCCTAT
TCCTTCAGCTATGATATCAAAGATGAGACAAGTGGAACGGATTTTTTCCGCTCTG
AAGAAAGCTCAGGCCCTGTGACGAAGGGAAGTTATAAGGTGGCTCTTCCTGATG
GTCGTATTCAAATTGTTGAATATATTGCAGATGAAAATGGCTATAAGGCCACAGT
TTCCTATGAGGGAGAAGCTGTTTTCCCAAATCCAGATGATTTTGAAGAAGAACCA
ACTCGGAGAACTTTTAGACACTCAAGAAAAGTCGATATTGACTCTGTACCCAATA
ACAACTACTCATTTTTGCGGAATAGAGTCCGTGGTGCAGGGACTACAGAAAAAG
CTCCTTCTCCTCAAGACACAACCATTCGTCCAGTCAGTCTTCCATTAAGACATCGA
TTATCTCGTGCCGAAGTCTCAAAATCACTTCNTACGAGTCCGTTTCCTTATGCTGT
GAGTAGTACGTCACCAGCTCCATTACCTTCTAGTCAAGGCCCACAACGTTTTCGT
GTTCATCATTCTTCACCAAATGTTGAGGGACGAGTATTACATCACTCAACACCTC
CAGTCAGCTATTCCCAACTCCCCCAAATCGCGACAACTCAAAAACCCAGATTCCT
TCATAACGCCAATTACGAAGAAGCATTACGAGACTATGGAATTAAGGTTGATTAT
***TAA***TACCTTTACTTTTTGTGATCCATTTAATATGGGTTATAAATATATTTATTAGT
ACTCATTGTTTATTTATTGTAAAAGCCGTTTAGTTGTTCAGATGAAAGTG

# FIG. 16

**Vitellogenin-like SL-0903**

FQRNMIRGWAQRLQLNMDKINNHGHGFHSEEQSIFGDCDTLYTVSDHKIVKSVSHTKDCKNRVHVLIDD
WRGERCDIDPEHPESRENPNGLYSASNTIYVVDKKGDHFHPKAIIGSSSVVAQFYQMEGVSFIAHSNST
SILKSVEDISEPMVVVGIPVKDLKYEFEDKEYQWNSDRDLKAREEHLSTGEFFESDMSTLSKYVKEKLN
KFHDIMQHLSNDKDAIAEAHDNGVNSMVPGMLAMDYNTLKAMSEELHSDKSDEGVFKYNLFNELLGSLG
TSASALLVRDMIMEDKFENFRDAVRALTAIPFHIRHPSKQLLSEFEALYNYDGDQLLKDAVPIVLGHLA
RVTCERAGVMHSPASKECFHSVVDGYADKTIEKIMGASDHKEQIKLLGMLFNLRYGNVAEKLKPLIYGE
TEIKCGHLRTLAVQAAAFGTINNGKGAEYLLPIFADSENSHELRLTALSYLMDAHPTATHFNTIVAVLY
REKDYEVINYAFTLLDKYATNINPCKKSVSVLAKYFLKYLKQYSHFETDYGLGVSKTYSRQFQQSKYGY
GGEYSYWVIGSHSSTLPLSVAMCMDTTLFGGYTANGMCVQLRIEGLSKALIRKFKTMSPDIWKSEDLKS
ILMGDMNIKERPDQPINVEVLLFVKNSVVAFRQYNEDSIKEGGNLKEIFDQLKGLGDTYSINHQRAMRF
GSLLYQQPLEVGAPVSYLNSFTGVFDVQATIKKGNARGLMFRDVKYNMNFFGHGSRMMMVQNPQSKMFY
SISQNRIYGSHFPREFVIGVNPLKKEFKLSIQRPSYENPLVLMMHSLTKVYTGSQNVNEKQDISANCPE
CKSDTPVSYGPDAAKTRVFLNHDCDKTGSYIHGEYFDCEMESNRGKVLYHLWRAMLPYNKNPKTFGNGI
RMGIRQIRAYFVFFPRAEKCGAMLRWSQSKENPVKELEISLRFNANPNGERLFFRGRKWVVTTIIKAKG
EPQDRVYKIILGHEFTPGYIENRLKFRMQRAAVPGIMSDYSICFNMENKYPDFGEEFMTYDKSTQLKMT
GKAKLQYGAAADCDSTPGEMKLSFKHETTEEAREAMKHTWYYEKCMEQKKQPEWANRGDKLPFTQACHM
TTWDATTARKYSWKMNFVKMTDRMNAIVSQFQSIMKTGLLPYWDIDPEIIPATSADPHMNIKATLKNHD
KNVDMYMETSQGGQRFNDIPLSLNWRPMLRNLKFTSTTRRLMQYKIIHGCTTTIDHVYTLDNVTYPYTP
TSCWTLASGHCSPHPTYAVFVKKSAGSHLDAKIYLGGHSIEFQTSGPKKINVLINGEAIDVGEEEHVHE
QDGQEIFKVLKWGSSYSVYSFLKIWVVYDGHAVSLIPAPSVTGQHCGLCGNFNRNQYDEFESKDAHQLK
TSDELVEDYKWKC

**BLAST hits:**

tr Q9NDN7 _TENMO Melanization-related protein [160 kDa MRP] [Tene... 147 2e-33
tr O17428 _9HYME Vitellogenin [Pimpla nipponica] 73 4e-11

# FIG. 17

**SEP protein 1 SL-0547**
MAKNKNVGKPRNYKLASGVVRFGKSKMYHKKAIYKFLKKTTPKKVEASKPAFVEK
KVGGAKNGGTRMVRVKKLKNDFPTMERRAHRIAKKPEKLSRRVRPTLTPGTIAVILA
GIHKGKRIVILKELSSGMLLISGPFKLNNCPIRRINQRYLLATSTKLDVSSIKMPENIND
DYFRRLRAAKKPAGSVFEGKKEEYKPSEQRKKDQVEVDKQLLNVIMKHPEASLLKQ
YLKKSFGLSKGQYPHNMKF

**BLAST hits:**
tr Q5UAT5 _BOMMO Ribosomal protein L6 [RpL6] [Bombyx mori (Silk m... 242 6e-63

# FIG. 18

**SEP protein 2 SL-0858**

**M**KIIAIFALLFIAVSGEDLEWESCNPDNLGEGDIALSPYPLPVVSGTSLDLKALFDL
HKDLDGDVDVELKLVKKGIVSIPIPCIESPSGLHLGSCSYKLEEIVSKYAYFLCPDY
FPEGQSCSFPLKAGQYGGEISGIVLPDIPPSISNLAKGTIHGTLSVTRNGEEVFCIN
GDLKMTN

**BLAST hits:**
tr Q5EN79 _9CNID Ganglioside M2 activator-like protein [Aurelia a... 44 0.001

# FIG. 19

## SEP protein 3 SL-1469

**M**RVTALLCLFVAAVSGSIFEDGKQYVFDSETSVVVGTMDHAPHSSGFAYKHHTTMQV
QGDNIKVKLSDVEFSQFNGKHENGEFPFDHTNFVATNRDIPAFEVQLDSHGLFSSLK
VGPKLTLFQRNMIRGWAQRLQLNMDKINNHGHGFHSEEQSIFGDCDTLYTVSDHKIV
KSVSHTKDCKNRVHVLIDDWRGERCDIDPEHPESRENPNGLYSASNTIYVVDKKGDH
FHPKAIIGSSSVVAQFYQMEGVSFIAHSNSTSILKSVEDISEPMVVVGIPVKDLKYE
FEDKEYQWNSDRDLKAREEHLSTGEFFESDMSTLSKYVKEKLNKFHDIMQHLSNDKD
AIAEAHDNEVNSMVPGMLAMDYNTSKAMSEELHSDKSDEGVFKYNLFNELLGSLGTS
ASALLVRDMIMEDKFENFRDAVRALTAIPFHIRHPSKQLLSEFEALYNYDGDQLLKD
AVPIVLGHLARVTCERAGVMHSPASKECFHSVVDGYADKTIEKIMGASDHKEQIKLL
GMLFNLRYGNVAEKLKPLIYGETEIKCGHLRTLAVQAAAFGAINNGKGAEYLLPIFA
DSENSHELRLTALSYLMDAHPTATHFNTIVAVLYREKDYEVINYAFTLLDKYATNIN
PCKKSVSVLAKYFLKYLKQYSHFETDYGLGVSKTYSRQFQQSKYGYGGEYSYWVIGS
HSSTLPLSVAMCMDTTLFGGYTANGMCVQLRIEGLSKALIRKFKTMSPDIWKSEDLK
SILMGDMNIKERPDQPINVEVLLFVKNSVVAFRQYNEDSIKEGGNLKEIFDQLKGLG
DTYSINHQRAMRFGSLLYQQPLEVGAPVSYLNSFTGVFDVQATIKKGNARGLMFRDV
KYNMNFFGHGSRMMMVQNPQSKMFYSISQNRIYGSHFPREFVIGVNPLKKEFKLSIQ
RPSYENPLVLMMHSLTKVYTGSQNVNEKQDISANCPECKSDTPVSYGPDAAKTRVFL
NHDCDKTGSYIHGEYFDCEMESNRGKVLYHLWRAMLPYNKNPKTFGNGXRMGIRQIR
AYFVFFPRAEKCGAMLRWSQSKENPVKELEISMRFNANPNGERLFFRGRKWVVTTII
KAKGEPQDRVYKIILGHEFTPGYIENRLKFRMQRAAVPGIMSDYSICFNMENKYPDF
GEEFMTYDKSTQLKMTGKAKLQYGAAADCDSTPGEMKLSFKHETTEEAREAMKHTWY
YEKCMEQKKQPEWANRGDKLPFTQACHMTTWDATTAP

**BLAST hits:**

tr Q9NDN7 _TENMO Melanization-related protein [160 kDa MRP] [Tene... 137 1e-30

Melanization-related protein [160 kDa MRP] [Tenebrio molitor (Yellow mealworm)]

sp P18948 VIT6_CAEEL Vitellogenin 6 precursor [vit-6] [Caenorhab... 67 2e-09

tr Q7KPP7 _CAEEL Vitellogenin structural genes (Yolk protein gene... 67 2e-09

tr Q8JIF9 _9GOBI Vitellogenin [Vg-530] [Acanthogobius flavimanus] 66 4e-09

# FIG. 20

**mussel adhesive protein SL-0927**

**M**KTSIIFSLYVLPSILHLAVSEDKTIIAEDLTAVESRYKVDAKPSPYVPPQPAPDFD
YFAPTVSPSFSPIASPSPSSPVTSYFTPTTIPPVTPSTTTTTVTTTTPATSTYRKLF
FPTSFKPSFLSSRKKLTTTTTTPATTSSTTTTITFTPTTSPPPSANEVRTTLNPSKV
ASKTKSYTRPLYSKKNFLRKKPSVYYKVKKNPVKLRKVKKILRPVSYSTPQTHPSST
TVSTTRDYPSKSLESLTQTKSPEIVSAFTPVSVSKKSIKSLNARKSSYASPSTPSFR
YSPTTPSSYQSLKPFEPKPIHRFRSKPGYKSTRSKPTYVSSTTTHPAYVSSTISPAY
ASSSVSPAYASSSVSPAYASTTVKPVFVSTTANEIYYTPEPKRVRSLPLTREQAHLY
SSIPYDSTTTSRQPPAPVSYSTPKPHSKQHSQYRELPLTREQSENIEFSTPVKANTK
PYNNNIQFNPVRRIPSHYRNHQALNEIRREEKYPAQPYSFSYDIKDETSGTDFFRSE
ESSGPVTKGSYKVALPDGRIQIVEYIADENGYKATVSYEGEAVFPNPDDFEEEPTRR
TFRHSRKVDIDSVPNNNYSFLRNRVRGAGTTEKAPSPQDTTIRPVSLPLRHRLSRTE
VSKSLPTSPFPYAVSSTSPAPLPSSQGPQRFRVHHSSPNVEGRVLHHSTPPVSYSQL
PQIATTQKPRFLHNANYEEALRDYGIKVDY

**BLAST hits:**
tr Q9V918 _DROME CG9036-PA (RE26879p) [CG9036] [Drosophila melano... 76 2e-12
tr Q5R283 _DROSI CG9036 [Drosophila simulans (Fruit fly)] 76 2e-12
tr Q5R279 _DROSE CG9036 [Drosophila sechellia (Fruit fly)] 76 2e-12
tr Q5TRT2 _ANOGA ENSANGP00000029010 (Fragment) [ENSANGG0000002361... 75 4e-12
tr Q9V6T6 _DROME CG6305-PA (GH20904p) [CG6305] [Drosophila melano... 74 1e-11
tr Q7QGL7 _ANOGA ENSANGP00000018947 (Fragment) [ENSANGG0000001645... 70 2e-10
tr Q9V7U0 _DROME CG15920-PA, isoform A [CG15920] [Drosophila mela... 70 2e-10
tr Q9NDT7 _BALAM BCS-1 [bcs-1] [Balanus amphitrite (Barnacle)] 61 8e-08

FIG. 21

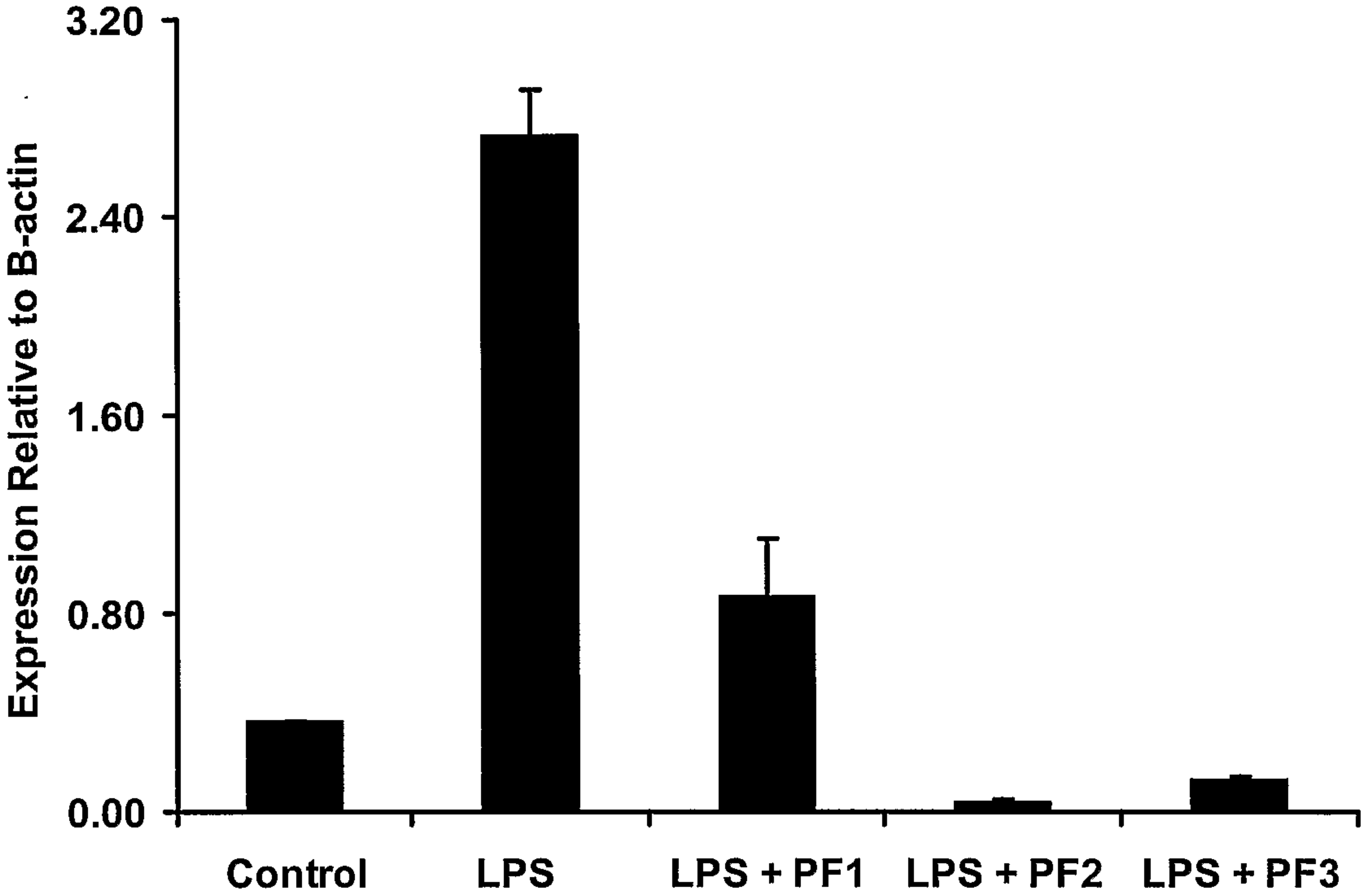
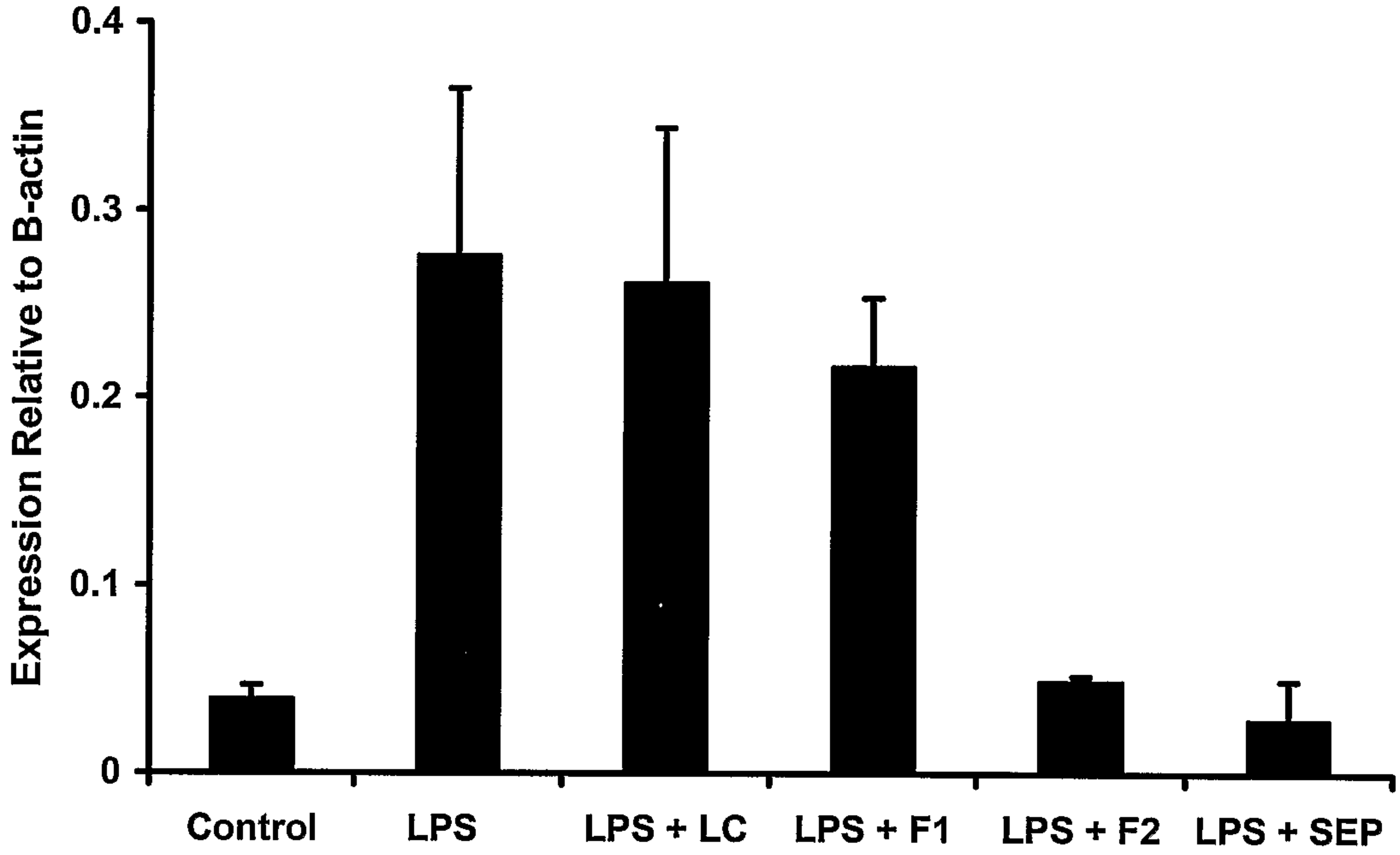
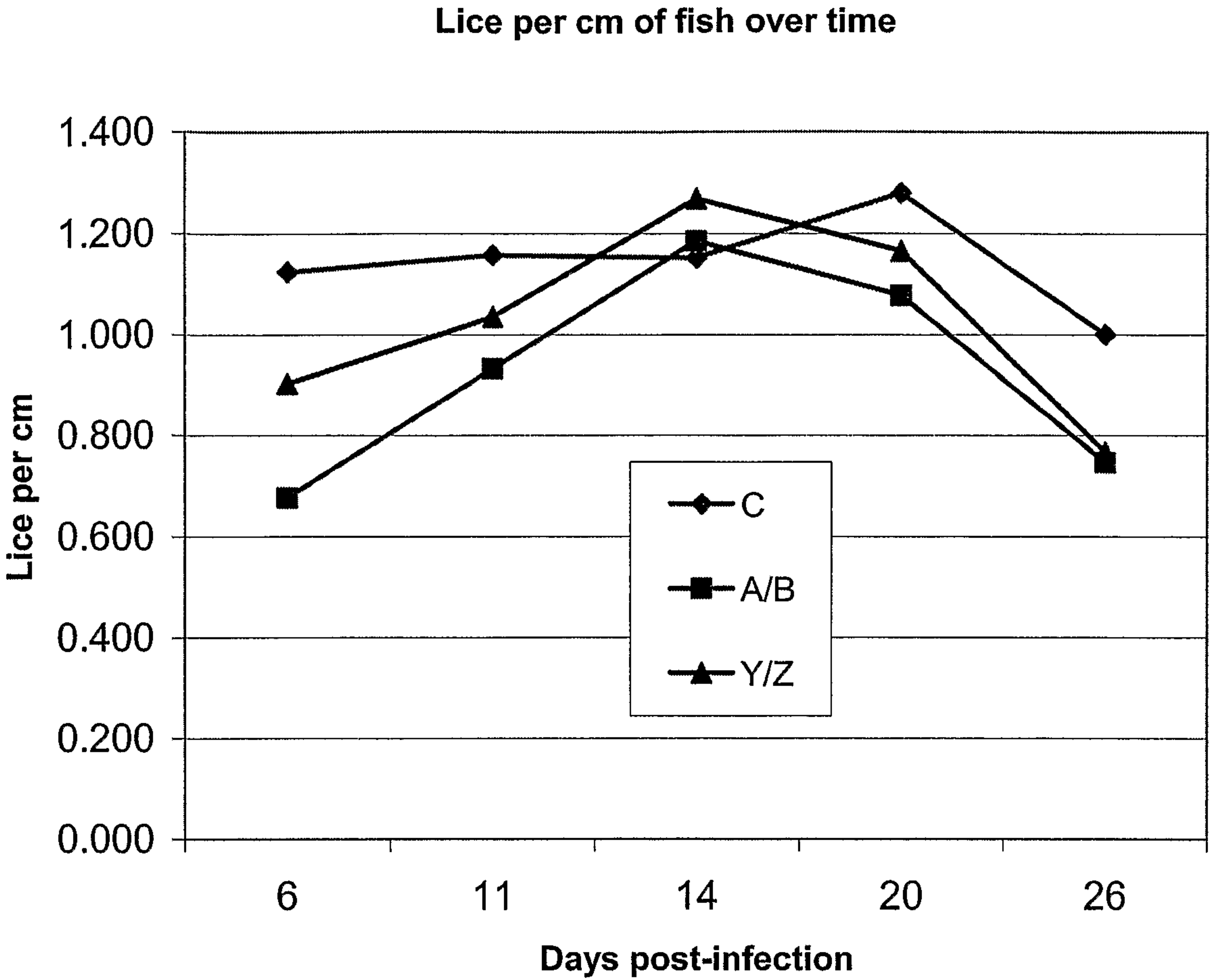

3.20
2.40
1.60
0.80
0.00
Expression Relative to B-actin
Control
LPS
LPS + PF1
LPS + PF2
LPS + PF3

FIG. 22


0.4
0.3
0.2
0.1
0
Expression Relative to B-actin
Control
LPS
LPS + LC
LPS + F1
LPS + F2
LPS + SEP


Lice per cm of fish over time
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Lice per cm
6
11
14
20
26
Days post-infection
C
A/B
Y/Z