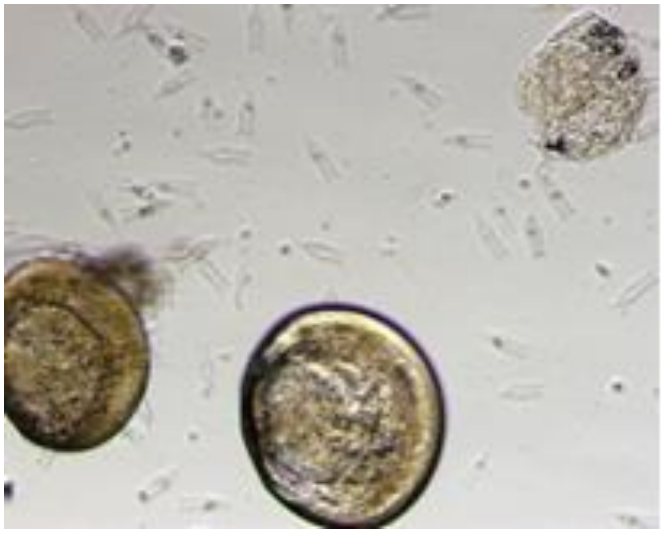
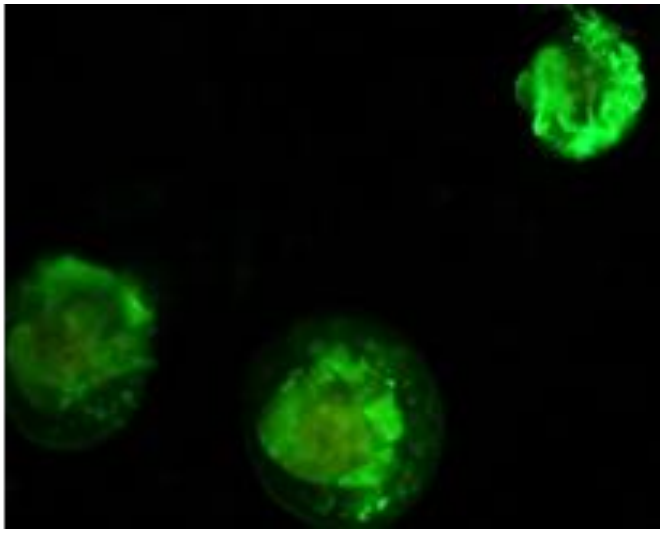
RECLAMATION

Managing Water in the West

Monoclonal Antibodies for Improved Detection of Quagga Mussel Larvae

Research and Development Office
Science and Technology Program
Program Priority: Mitigating the Impact of Invasive Mussels on Water and Power Operations
Final Report ST-2014-9640-01





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Quagga and zebra mussels (*Dreissena* spp.) are aquatic, invasive, bivalve species that cause considerable damage to submerged infrastructure involved in the conveyance, treatment, storage, and use of water. Among Bureau of Reclamation reservoirs, quagga mussels (*Dreissena bugensis*) were first detected in Lake Mead in 2007. Following initial introduction into an aquatic system, mussels attach to most submerged surfaces, with serious consequences for the drinking water and hydroelectric power industries, industrial cooling facilities, agricultural irrigation, and recreational use of water. Consequently, it is critically important to detect infestation in the early stages so that timely and cost-effective response plans and control strategies can be developed. The goal of this project was to develop a monoclonal antibody (mAb) that can be used to label veligers with fluorescent tags and purify veligers from complex samples using magnetic capture technology. Organism-specific antibodies have previously been used to improve detection and identification of many organisms in environmental waters, including mussel larvae. An antibody that recognizes quagga mussel veligers, coupled to magnetic beads or fluorescent tags, will greatly improve microscopic detection and identification of veligers in aquatic systems.

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Monoclonal Antibodies for Improved Detection of Quagga Mussel Larvae

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Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Abbreviations and Acronyms

µg	microgram
µm	micrometer
ATCC	American Type Culture Collection
cm	centimeter
CPLM	cross-polarized light microscopy
CRA	Colorado River Aqueduct
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
FITC	fluorescein isothiocyanate
IgG	immunoglobulin G
kDa	kilodaltons
L	liter
mAb(s)	monoclonal antibody(ies)
min	minute
mL	milliliter
mM	millimolar
MSE	modified saline ethanol
NaCl	sodium chloride
nm	nanometer
OD	optical density
pAb(s)	polyclonal antibody(ies)
PBS	phosphate buffered saline
RT	room temperature
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
VFD	variable frequency drive

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Executive Summary

Quagga and zebra mussels (*Dreissena* spp.) are aquatic, invasive, bivalve species that cause considerable damage to submerged infrastructure involved in the conveyance, treatment, storage, and use of water. Since their initial introduction into the United States in the 1980s, they have caused significant problems for utilities and industries in many Eastern and Central States, particularly in the Great Lakes region and along the Missouri and Mississippi Rivers. Quagga mussels (*Dreissena bugensis*) were first detected in Lake Mead in 2007. Since then, the mussels have spread downstream into the Lower Colorado River region and have invaded the Central Arizona Project and the Colorado River Aqueduct (CRA), including Lake Mathews, the terminal reservoir on the CRA. Following initial introduction into an aquatic system, mussels attach to most submerged surfaces, with serious consequences for the drinking water and hydroelectric power industries, industrial cooling facilities, agricultural irrigation, and recreational use of water. Colonies of mussels clog intake trashracks, pipes, valves, siphons, and irrigation and fire-suppression systems. Consequently, it is critically important to detect infestation in the early stages so that timely and cost-effective response plans and control strategies can be developed.

The mussel life cycle includes microscopic, planktonic larval stages (veligers) that are typically detected using cross-polarized light microscopy (CPLM) on plankton net concentrates; veligers have a distinct cross pattern due to birefringence caused by calcium carbonate in their shells. However, other bivalve larvae may produce similar patterns and be confused for quagga or zebra mussel veligers. In addition, concentrated water samples usually contain many other organisms and debris that can interfere with veliger detection. Therefore, methods are needed to simplify and improve veliger detection to ensure maximum confidence in the results of monitoring programs.

The goal of this project was to develop a monoclonal antibody (mAb) that can be used to label veligers with fluorescent tags and purify veligers from complex samples using magnetic capture technology. Organism-specific antibodies have previously been used to improve detection and identification of many organisms in environmental waters, including mussel larvae. An antibody that recognizes quagga mussel veligers, coupled to magnetic beads or fluorescent tags, will greatly improve microscopic detection and identification of veligers in aquatic systems.

Polyclonal antibodies (pAb) are easier, quicker, and less costly to produce, compared to mAb, so pAb were initially produced to assess the feasibility of antibody production using different preparations of veliger and adult mussel tissue. Examination by epifluorescence microscopy demonstrated intense fluorescence in all stained samples, with the strongest fluorescent signals concentrated around the exposed vellum tissues near the opening of the bivalve veliger shell. Microscopic observation following immunomagnetic capture of

veligers from Lake Havasu also showed localized binding of the pAb-magnetic bead complex to exposed veliger vellum tissue. Recovery efficiencies for pAb-mediated magnetic capture were 48 – 96%. However, pAbs can contain antibodies to multiple antigens that were present in the original immunogen so they often lack specificity. Therefore, having demonstrated the feasibility of antibody production, the project focused on producing an anti-quagga mAb, which should have higher specificity because it is a single antibody recognizing a single antigen, rather than a mix of antibodies recognizing multiple antigens.

To generate mAbs, protein was extracted from the mantle tissue of adult mussels harvested from Lake Havasu and the Colorado River Aqueduct, and used as the antigen to elicit an initial antibody response. Protein extracted from 51 mussels covering four size classes and three locations was combined to produce a single heterogeneous immunogen. At the end of the 10-week immunization period, antibody-producing spleen cells were fused to each of two myeloma cell lines to produce fusion hybridomas, which were grown in 96-well plates using standard cell culture procedures. Producing a mAb requires multiple rounds of generating and screening many cell cultures. All of the fusion products were screened against the original protein antigen using an enzyme-linked immunosorbent assay (ELISA) to determine whether they produced antibodies. Cell culture supernatants from the 10 hybridomas that displayed the strongest ELISA signal were also screened against whole quagga mussel veligers by indirect immunofluorescence microscopy. Based on the intensity of their fluorescence, six antibody-producing cell lines were subcloned to produce 20 cell lines. Cell culture supernatants containing unpurified antibody from 20 subcloned hybridoma cell lines were screened against protein extracted from adult quagga mussels and *Corbicula* using an ELISA assay. Most cell culture supernatants had relatively high absorbance values when assayed by ELISA against quagga mussel protein compared to *Corbicula* protein, indicating a high level of specificity for quagga-specific antigen(s). All of these antibodies were isotypes IgG1κ or IgG2bκ.

Twenty mAbs were produced as unpurified reagents, and eight were produced and purified on a larger scale, yielding milligram quantities of purified reagent grade mAb. When coupled with a secondary fluorescently labeled antibody, these quagga-specific antibodies stained veligers, generating a variety of fluorescence staining patterns when observed by immunofluorescence microscopy. Some of the antibodies stained the entire internal veliger contents, generating intense green fluorescence, while others produced a speckled pattern of fluorescence with multiple foci of intense staining against an overall background of less intense staining. In other cases, staining was focused in the extruded vellum at the shell opening and around the periphery of veligers, while other subclones had intense staining that was localized to a single internal region of the veliger, which may represent a particular organ or part of an organ.

Recovery efficiencies for paramagnetic beads coated with unpurified mAb from cell culture supernatants or purified mAb were not as high as those achieved with pAb. These low recovery efficiencies may be due to poor binding of the paramagnetic beads to the primary anti-quagga antibody or insufficient or inappropriate blocking of nonspecific binding sites. It is also possible that the antigenic binding sites are inside veligers at locations that are inaccessible to the relatively large, antibody-coated, paramagnetic beads. The low recovery efficiencies demonstrate that additional evaluation and optimization of mAb-mediated immunomagnetic capture is needed.

Overall, the project was successful. Additional specificity testing is necessary, along with testing the performance of mAbs against veligers in more complex matrices. In addition, continued development of the immunocapture technique is required. Nevertheless, the antibodies produced by this project provide tools that could simplify microscopic detection and identification of veligers in water samples.

1. Introduction

Quagga and zebra mussels (*Dreissena* spp.) are aquatic, invasive, bivalve species that cause considerable damage to submerged infrastructure involved in the conveyance, treatment, storage, and use of water. Quagga mussels (*Dreissena bugensis*) were first detected in the Western United States in Lake Mead in 2007. Since then, the mussels have spread downstream into the Lower Colorado River region and have invaded the Central Arizona Project and the Colorado River Aqueduct (CRA), including Lake Mathews, which is the terminal reservoir on the CRA (Anderson and Taylor, 2011; Reid et al., 2010).

Following initial introduction into an aquatic system, mussels attach to most submerged surfaces. Affected sectors include the drinking water industry, hydroelectric power, industrial cooling facilities, agricultural irrigation, and recreational use of water. Colonies of mussels clog intake trash racks, pipes, valves, siphons, irrigation, and fire-suppression systems. Consequently, it is critically important to detect infestation in the early stages so that timely and cost-effective response plans and control strategies can be developed.

The mussel life cycle includes microscopic, planktonic larval stages called veligers, which have a diameter of 50-350 micrometers (µm) (figure 1A). These veligers are typically detected using cross-polarized light microscopy (CPLM) after plankton samples are collected (using 63-µm mesh nets) and concentrated by filtration and centrifugation or settling. Most early warning monitoring programs target these larval stages; however, concentrated samples of environmental water typically contain many nontarget organisms, such as copepods, diatoms, rotifers, cyanobacteria, and algae. Samples can also include larval stages of unrelated mussels and clams. When observed under cross-polarized light, veligers have a distinct cross pattern due to birefringence caused by calcium carbonate in their shells (figure 1B). However, a variety of factors can interfere with veliger detection by CPLM. For example, the stability of veliger birefringence is affected by sample preservation conditions (O'Meara et al., 2013). Other organisms with calcium carbonate based shells may produce similar birefringence patterns and be confused for quagga or zebra mussel veligers. In addition, the high density of nontarget organisms and debris in water concentrates can interfere with veliger detection. Therefore, tools and methods are needed to simplify and improve veliger detection to ensure maximum confidence in the results of monitoring programs.

Sensitive molecular methods have been developed to detect quagga and zebra mussel veligers and other invasive mussels (Frischer et al., 2012; Ludwig et al., 2014; Rochelle et al., 2010), but they are most useful as early-warning monitoring tools. Direct observation by microscopy is still the most appropriate approach for monitoring sites that are already infested and assessing the level of infestation.

The goal of this project was to develop antibodies that can be used to improve microscopic detection methods. These antibodies will be used to purify veligers from complex environmental water samples using magnetic capture technology and to label veligers with fluorescent tags. Fluorescently labeled organisms are much easier to detect and enumerate by microscopy. Organism-specific antibodies have been used in many fields to aid in the isolation of organisms from complex samples and to label those organisms with fluorescent tags, thus aiding detection and identification. For example, the U.S. Environmental Protection Agency (EPA) approved method, which is used nationwide for detecting the protozoan parasites *Cryptosporidium* and *Giardia* in water, uses antibodies for immunomagnetic purification and detection by fluorescence microscopy (EPA, 2012). Of more direct relevance to the current project, monoclonal antibodies have been used for detection and identification of larvae of the economically important mussel *Mytilus galloprovincialis* (Pérez et al., 2009).

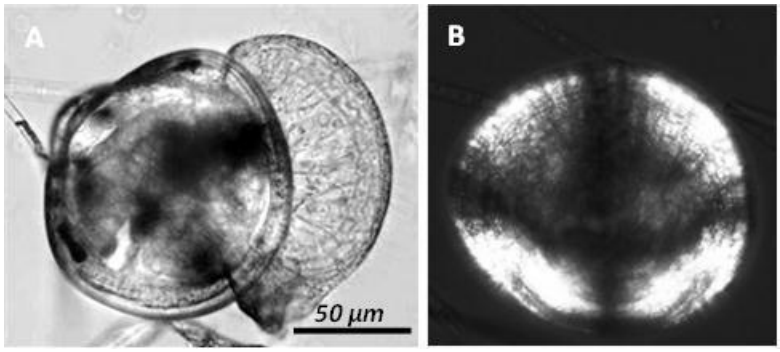

Figure 1. Quagga mussel veligers viewed by microscopy: (A) white light image of an umbonal veliger with vellum extended beyond the shell perimeter; (B) cross-polarized light image of a different umbonal veliger.

An antibody that specifically recognizes quagga mussel veligers, coupled to magnetic beads or fluorescent tags, will greatly improve microscopic detection and identification of veligers in aquatic systems. The characteristics of this antibody should include specificity for quagga mussel veligers, no recognition or binding of nontarget organisms, recognition of an abundant protein antigen that is expressed on the outside surface of veligers, and high binding strength (avidity) to ensure that the antibody stays attached during laboratory manipulations.

While the antibody will ideally be specific only for quagga mussels, it will likely also cross-react with zebra mussels. Quagga and zebra mussels are relatively closely related and probably share many protein similarities. However, antibodies that are specific for quagga mussels alone, or both quagga and zebra mussels, will be equally useful for continued research and methods development.

1.1 Antibody Structure

Most animals produce IgG antibodies in response to an immunogenic stimulus, and the overall chemical structure of antibodies is similar across animal species. Recognition and binding of antigens occurs at the end of the variable Fab fragments of IgG. Mouse antibodies are classified as IgG1, IgG2a, IgG2b, or IgG3, and each of them can have either a kappa (κ) or lambda (λ) light chain (figure 2). The nonvariable Fc fragment of IgG antibodies is often used in laboratory procedures as a binding site for secondary antibodies or to attach the antibody to magnetic beads.

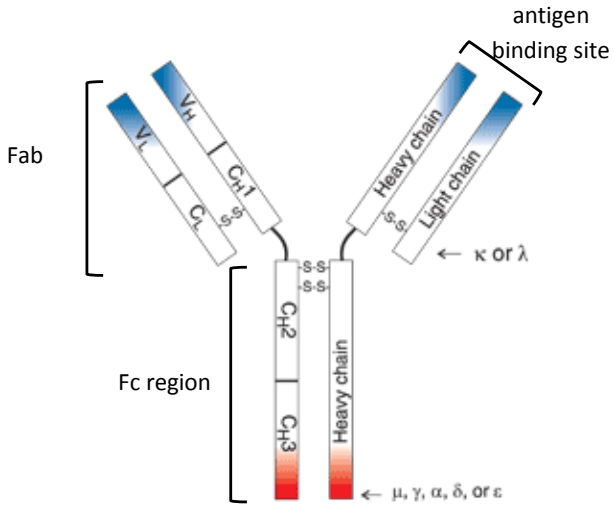

Figure 2. Structure of IgG antibody.

Polyclonal antibodies can be generated relatively quickly and inexpensively; however, they contain a mixture of antibodies that target many different antigenic sites on the target organism, as well as on other organisms that may have been present in the original sample used for immunization. Therefore, the level of specificity of polyclonal antibodies is highly dependent on the purity of the original antigenic preparation. While this increases the likelihood that at least some of the antibodies will recognize the target organism, it also increases the likelihood of nonspecific binding to nontarget organisms. Also, every time a new batch of polyclonal antibodies is produced, it can contain a different mixture of antibodies that may recognize different antigenic sites on the target organism.

In contrast with a polyclonal antibody, a monoclonal antibody (mAb) is a single antibody that recognizes a single antigen and, therefore, can potentially be much more specific than polyclonal antibodies. The animal cell that produces the specific mAb is fused to an immortal cell line that can be grown in large quantities in a laboratory and stored indefinitely to ensure a continual supply of

the same specific antibody. However, the production, screening, and evaluation processes for a mAb require substantially more time and effort compared to polyclonal antibodies. There is no structural difference between monoclonal and polyclonal antibodies.

2. Preliminary Work with Polyclonal Antibodies

Polyclonal antibodies were initially produced to assess the feasibility of antibody production using veligers and adult mussel tissue. Antibodies were produced by QED Bioscience, Inc., in San Diego, California, using frozen laboratory-bred veligers, crude protein extracted from these veligers, and adult mussel mantle proteins prepared at Metropolitan Water District of Southern California's (MWDSC) Water Quality Laboratory. Two polyclonal antibodies (pR1000 and pR1030) were produced, as described in previous progress reports submitted to the Reclamation S&T Program. At various stages in the antibody production process, crude rabbit antiserum was tested to ensure that immunization produced antibodies bound to veligers. Subsequently, the antibody was purified by Protein-G affinity chromatography. Both antibodies were used to assess fluorescent staining and immunomagnetic capture of veligers. Veligers were fixed with methanol or ethanol and incubated in blocking buffer (2% goat serum in PBS and Tween-20) to reduce nonspecific antibody binding. They were stained in solution in a Sedgwick Rafter chamber with unlabeled purified antibody (pR1000 or pR1030), followed by anti-rabbit IgG conjugated to a fluorescent Texas Red label (secondary antibody). Examination by epifluorescence microscopy revealed intense fluorescence in all stained samples, with the strongest fluorescent signals concentrated around the exposed vellum tissues near the opening of the bivalve veliger shell (figures 3 and 4). Grey/white areas in the fluorescence microscopy images are "photo-bleached," due to the high level of fluorescence, which indicates a relatively high concentration of bound polyclonal antibodies. Unstained controls displayed autofluorescence but less intensely than antibody-stained veligers. Microscopic observation following immunomagnetic capture of veligers from Lake Havasu also showed localized binding of the polyclonal antibody-magnetic bead complex to exposed vellum tissue (figures 5 and 6). Recovery efficiencies for polyclonal antibody-mediated magnetic capture were 48-96%.

The specificity of polyclonal antibody pR1030 was assessed by immunofluorescence microscopy on plankton net concentrates from the Sacramento River that contained *C. fluminea* larvae. The primary anti-quagga antibody was applied to sample concentrates fixed in 25% ethanol, followed by a secondary Texas Red-labeled goat anti-rabbit IgG. Fluorescence was observed around the edge of the larval shell (figure 7), but staining was not as intense when compared to quagga mussel veligers. Nevertheless, the results indicate some cross-reactivity of pR1030 with *Corbicula*, as might be expected with broadly specificity polyclonal antibodies.

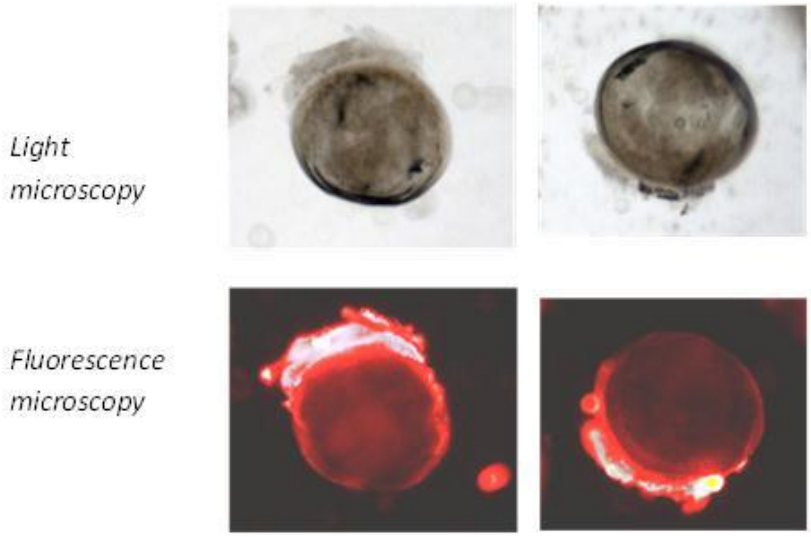

Figure 3. Indirect immunofluorescent staining of quagga mussel veligers with pR1000 polyclonal anti-veliger antibody and Texas Red-conjugated anti-rabbit IgG (fluorochrome excitation at 550 nm, emission at 615 nm). Veligers were collected from Lake Havasu using a 63-µm plankton net and were preserved in ethanol.

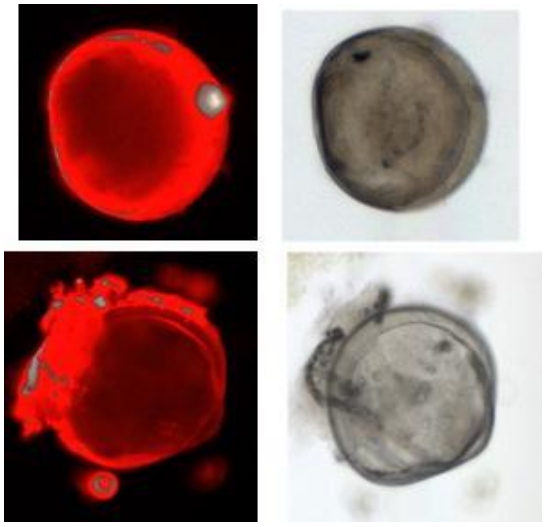

Figure 4. Left panel: Indirect immunofluorescent staining of quagga mussel veligers with pR1030 polyclonal antibody and Texas Red-conjugated anti-rabbit IgG (fluorochrome excitation at 550 nm, emission at 615 nm). Right panel: Light microscopy image of same veliger shown in panel A. Veligers were collected from Lake Havasu using a 63-µm plankton net and were preserved in ethanol.

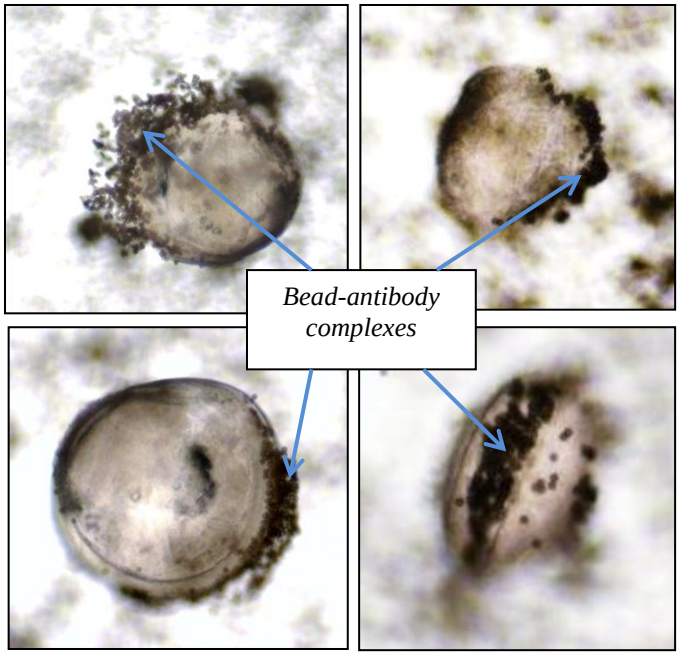

Figure 5. Light microscopy images of paramagnetic bead-pR1000 polyclonal antibody conjugates bound to veligers.

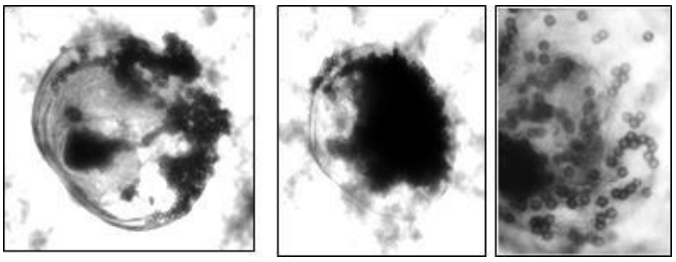

Figure 6. Light microscopy images showing paramagnetic bead-pR1030 polyclonal antibody conjugates bound to veliger vellum tissues and shells. The two panels on the left show entire veligers, while the panel on the right displays a higher magnification image of a portion of a veliger shell.

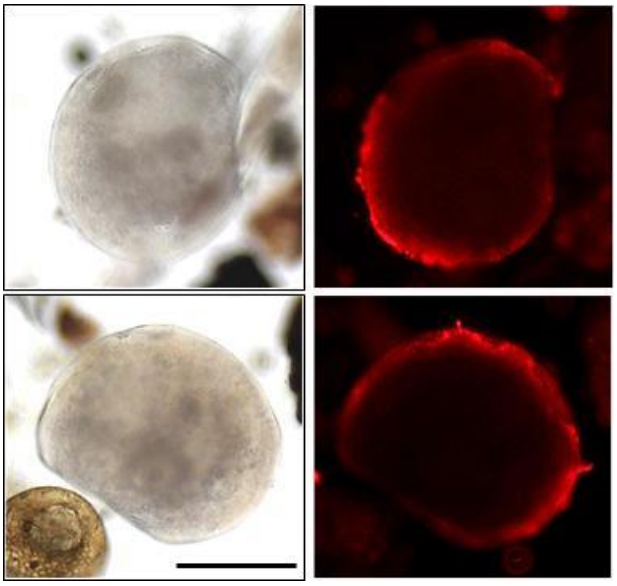

Figure 7. Indirect immunofluorescent staining of Corbicula larvae with pR1030 polyclonal anti-veliger antibody and Texas Red-conjugated anti-rabbit IgG (fluorochrome excitation at 550 nm; emission at 615 nm). Scale bar = 100 µm.

3. Monoclonal Antibody Procedures

The feasibility of producing antibodies that recognize and bind to quagga mussel veligers was demonstrated in the work described above with polyclonal antibodies. Therefore, this project focused on producing potentially more specific and more consistent mAb. Monoclonal antibodies should display greater specificity because they will be single antibodies recognizing a single antigen, rather than a mix of antibodies recognizing multiple antigens, as with polyclonal antibodies.

3.1 Adult Mussel and Veliger Collection

Adult mussels were collected from Lake Havasu and two raw water lakes within the Colorado River Aqueduct system by scuba divers. Veligers were collected from Lake Havasu by filtering approximately 1,000 liters of lake water through a plankton net (63-µm mesh). The concentrate was further concentrated by centrifuging for 15 minutes at 4,500 × g and aspirating the supernatant to give a final volume of ~5 milliliters (mL). Veligers were fixed in 25% ethanol for 30 minutes and then washed and resuspended in phosphate buffered saline (PBS). For some experiments, veligers were also resuspended in modified saline ethanol (MSE) containing 69% ethanol, 0.5 M Tris-HCl, pH 7.8, 0.35 M NaCl, and 2.5 mM EDTA. Veliger samples were stored at 4 degrees Celsius (ºC).

3.2 Protein Extraction

The strongest immune response in the polyclonal stage of the project was elicited by protein extracted from adult mussel tissue, so the same approach was used to generate mAb. A mAb raised against protein from geographically distinct mussels might not react with mussels or veligers from other locations, depending on the uniqueness or conservation of the antigen that is recognized by the antibody. Also, using protein from a single mussel size or age class might limit recognition of juvenile and larval stages. Therefore, protein was extracted from the mantle tissue of adult mussels collected from three locations (Lake Havasu and two locations within MWDSC's raw water system) using a total protein extraction kit (EMD Millipore, obtained from Billerica, Massachusetts). Great care was taken during mussel dissection to ensure that only mantle tissue was removed for protein extraction. It was particularly important to avoid veliger gut contents such as algae, cyanobacteria, other bacteria, and protozoa so that an antibody was not generated against a spurious nontarget organism. Protein was also extracted from adult *Corbicula fluminea* mantle tissue (harvested from Lake Mathews, California) as a control for testing antibody specificity. For each location, protein was extracted from multiple mussels in four size classes covering 1 to 3 centimeters. Extracted protein was quantified using a modified Lowry

protein assay (DC Protein Assay, obtained from BioRad, in Hercules, California) with absorbance measured at 750 nm using a Synergy Mx spectrophotometer (obtained from BioTek, in Winooski, Vermont) and reference to bovine gamma globulin standards. Yields were 0.32 to 2.22 milligrams (mg) of protein per group of mussels (three to six mussels per group). Extracted protein from each mussel was then mixed together and stored at -80 °C. The resulting immunogenic mixture contained protein from 51 wild adult mussels from 3 locations, to maximize the likelihood of generating mAb that recognize useful markers in veligers.

Analysis of the total extracted protein by polyacrylamide gel electrophoresis demonstrated similar fractionation patterns for quagga and *Corbicula* proteins (figure 8). The protein extraction kit produced the final protein in a buffer containing a low concentration of detergent and preservative. These additives may interfere with the immunogenicity of the protein or interfere with antibody production following immunization; therefore, the combined extracted protein was purified by dialysis against PBS using a 3.5 to 5 kiloDaltons (kDa) molecular weight, cutoff dialysis membrane. The final yield of purified protein was 5.7 mg, which was diluted in PBS to 1 milligram per milliter (mg/mL).

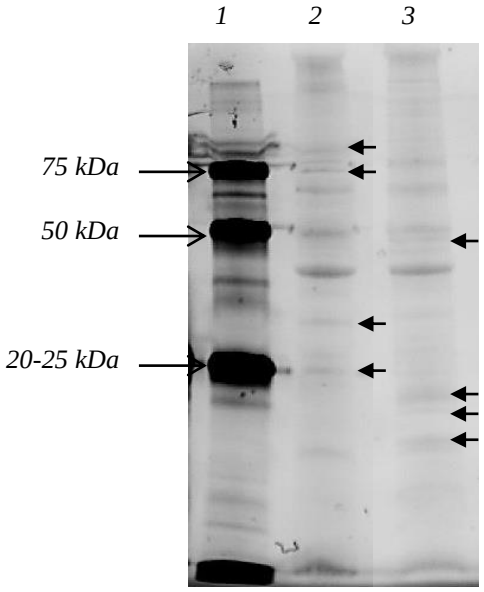

Figure 8. Electrophoretic protein profiles from Dreissena bugensis (lane 2) and Corbicula fluminea (lane 3). Primary differences are indicated by arrows. Lane 1 contained protein size standards.

3.3 Initial Monoclonal Antibody Production

Frozen extracted protein was shipped to QED Bioscience, Inc. (located in San Diego, California) on dry ice via overnight courier. Initially, five Swiss Webster mice were immunized with 50 micrograms (µg) of protein. Following a 4-week rest period, a booster immunization was provided by injecting 25 µg of protein suspended in incomplete Freund's adjuvant. Serum antibody titers in response to this immunization were 56,300 to 83,800. The serum antibody endpoint titer is defined as the highest serum dilution that still gives a positive response (optical density ≥0.1) in an enzyme-linked immunosorbent assay (ELISA) titered against the immunizing protein. Follow-up booster immunizations were performed at 2-week intervals with 5 to 10 µg of protein suspended in PBS. Serum antibody titers increased to 66,600 to 209,900 in the second bleed (approximately 6 weeks after initial immunization). The serum also reacted to *Corbicula* protein extracts (titer range of 60,200 to 171,500); however, at this stage in the process, the antibodies were still polyclonal, so a certain level of cross-reactivity was expected. The ratio of *Corbicula* to quagga signal was 0.7 to 0.9. Selection of monoclonal antibodies occurs at the later hybridoma stage.

Following a total of four booster immunizations over 6 weeks, antibody titer was assessed by ELISA against the original protein antigen. Antibody producing spleen cells from the sample with the highest ELISA titer were split into equal volumes and fused to each of two B lymphoblast myeloma cell lines originating from BALB/c mice (FO, ATCC CRL-1646 and P3X63Ag8U.1, ATCC CRL-1597). The resulting fusion hybridomas were seeded into three 96-well plates for each fusion partner, for a total of six 96-well plates, and then grown using standard cell culture procedures. The remaining bulk fusion cocktails were stored at -80 °C. All of the fusion products were screened by ELISA against the original protein immunogen to determine if they produced antibodies. A total of 112 cell clones were positive, and cell culture supernatants from the 10 cell clones that displayed the strongest ELISA signal were selected for further evaluation against whole veligers.

Hybridoma cell culture supernatants were screened against veligers by indirect immunofluorescence microscopy using a goat anti-mouse IgG-fluorescein isothiocyanate (FITC) conjugate (obtained from Sigma, located in St. Louis, Missouri) that bound to mAb in the cell culture supernatants. Most of these initial clones displayed very little or no reactivity with *Corbicula* protein. Of the original 10 positive hybridomas, 6 were expanded (subcloned), based on the intensity of their fluorescence. These 6 hybridomas were subcloned and cultured to produce 20 antibody-producing subclones. Cell culture supernatants from 20 subcloned hybridoma cell lines were screened against protein extracted from adult quagga mussels and *Corbicula* using an ELISA assay. At this stage, the antibodies were monoclonal but not purified. Extracted protein was bound to the wells of a 96-well plate and then used to capture the antibody from each cell culture supernatant. The complex was then detected with a peroxidase-labeled

secondary antibody and peroxidase-specific substrate. The resulting color change was proportional to the concentration of antibody in the supernatant, which was measured photometrically, and the result presented as optical density (OD) units. Most of cell culture supernatants had relatively high OD values when assayed by ELISA against quagga protein (>0.3 OD units), compared to ≤0.002 for *Corbicula* protein (table 1; ranked by Corbicula:Quagga [C:Q] ratio). A C:Q ratio of <1% indicates a relatively high level of specificity because the antibodies bound far more strongly to quagga protein than to *Corbicula* protein. Conversely, a C:Q ratio of >10% indicates relatively strong binding to *Corbicula* protein.

Table 1. Characteristics of Anti-Quagga Mussel Monoclonal Antibodies Produced by Hybridoma Subclone Cell Culture Supernatants, Ranked by Decreasing Specificity (increasing C:Q ratio)

Hybridoma Subclone	Isotype/ Subclass	ELISA Response (OD units)[a]		C:Q Ratio (%)[b]	Assessment of Veliger Staining by Immunofluorescence Microscopy[c]
		Quagga	*Corbicula*		
2E10-1D6	IgG2b k	0.371	0.001	0.27	Strong distinct staining of internal structures
2E10-1A8*	IgG2b k	0.369	<0.001	<0.27	Strong distinct staining of internal structures
2E10-1C2	IgG2b k	0.366	0.001	0.27	Strong distinct staining of internal structures
2E10-1B10*	IgG2b k	0.353	<0.001	<0.28	Strong distinct staining of internal structures
2E10-1A7	IgG2b k	0.349	<0.001	<0.29	Strong distinct staining of internal structures
2E10-1E12*	IgG2b k	0.342	<0.001	<0.29	Strong distinct staining of internal structures
2E10-1E4	IgG2b k	0.329	0.001	0.30	Strong distinct staining of internal structures
1B8-2C12*	IgG2b k	0.319	<0.001	<0.31	Strong distinct staining of internal structures
3C4-2B11*	IgG1 k	0.252	<0.001	<0.40	Bright staining around edges of veligers, weaker overall staining
2E10-1E3	IgG2b k	0.344	0.002	0.58	Strong distinct staining of internal structures
2E10-1G5	IgG2b k	0.344	0.002	0.58	Weak staining of internal structures
2E8-1C5*	IgG2b k	0.329	0.002	0.61	Strong distinct staining of internal structures
2E10-1G4	IgG2b k	0.308	0.002	0.65	Intermediate staining
2E10-1D5	IgG2b k	0.376	0.003	0.80	Intermediate staining
2E8-1C4	IgG2b k	0.111	<0.001	<0.90	Strong distinct staining of internal structures
3C4-2F5*	IgG1 k	0.102	<0.001	<0.90	Speckled but strong staining pattern
3E9-2C6	IgG1 k	0.185	0.019	10.27	Distinct localized internal staining
3E9-2C10	IgG1 k	0.259	0.057	22.01	Distinct localized internal staining
4E12-2H10	IgG1 k	0.323	0.186	57.59	Distinct localized internal staining
4E12-1G4	IgG1 k	0.348	0.205	58.91	Distinct localized internal staining

[a] Cell culture supernatants were screened by ELISA against proteins extracted from adult mussels.
[b] Specificity is inversely proportional to C:Q ratio; C:Q ≤1% indicates high specificity.
[c] Tested in an indirect immunofluorescence microscopy assay using veligers from Lake Havasu.
* Subclones selected for scaled-up production and purification.

All six of the hybridomas that were subcultured produced at least one antibody-producing subclone, although most of the 20 subclones were from the 2E10 parent hybridoma (55%, table 1). All of the antibodies were either IgG1κ (6 out of 20) or IgG2bκ (14 of 20) isotypes. Mouse antibodies are classified as IgG1, IgG2a, IgG2b, or IgG3, and each of them can have either a kappa (κ) or lambda (λ) light chain. In humans, the ratio of κ to λ light chain antibodies is 2:1. However, in mice the ratio is 20:1, so the majority of mouse antibodies, including custom monoclonal antibodies such as those produced for this project, will have κ light chains. The two types of light chain are never mixed in a single antibody. Classification as either κ or λ light chain is based on small polypeptide structural differences, but there is no functional difference between the two types of light chain. IgG3 is typically the least responsive IgG subclass in mice (typically less than 5% of total IgG population), so it is not surprising that none of the hybridomas for this project produced an IgG3 antibody.

3.4 Screening Cell Culture Supernatants by Immunofluorescence Microscopy

The 20 hybridoma subclones were screened against Lake Havasu veligers using indirect immunofluorescence microscopy. Veligers were stained with 500 microliters (µL) of cell culture supernatant, and the veliger-primary monoclonal antibody complex was detected with a secondary goat anti-mouse IgG conjugated to FITC (obtained from Sigma, located in St. Louis, Missouri) using the following procedure:

1. Place veligers in 1.7-mL microfuge tubes and centrifuge at 4,500 × g for 4.5 minutes at room temperature (RT).

2. Remove supernatant, resuspend in 1 mL of protein-free T20 blocking buffer (PBS plus 0.05% Tween-20, obtained from Thermo-Fisher, located in Rockford, Illinois), and incubate at RT for 15 minutes.

3. Centrifuge, remove supernatant, and resuspend in 500 µL of T20 buffer.

4. Add 500 µL of hybridoma cell culture supernatant and incubate for 2 hours at RT on a rotator.

5. Centrifuge sample (4,500 × g, 4.5 minutes, RT), remove supernatant, and resuspend in 1-mL buffer. Repeat.

6. Resuspend in 1 mL of protein-free T20 blocking buffer and add 10 µL of goat anti-mouse IgG-FITC (Sigma).

7. Incubate for 1 hour at RT on rotator in the dark, centrifuge sample and remove supernatant. Resuspend in 1-mL buffer and repeat.

8. Add 1 drop of mounting media containing anti-fade agent (obtained from Waterborne, located in New Orleans, Louisiana) to the sample to stabilize the fluorescence.

9. Observe by epifluorescence microscopy with excitation at 485 nm and emission at 515 to 565 nm.

3.5 Immunocapture Using Cell Culture Supernatants

Hybridoma cell culture supernatants were also evaluated for their capacity to capture Lake Havasu veligers when attached to paramagnetic beads (immunocapture) using the procedure described below.

1. Place veligers in 1.7-mL microfuge tube and centrifuge (4,500 × g, 4.5 minutes, RT).

2. Remove supernatant and resuspend in 1 mL of protein-free T20 blocking buffer (obtained from Thermo-Fisher). Incubate at RT for 15 minutes.

3. Centrifuge, remove supernatant, and resuspend in 500-µL T20 buffer.

4. Add 500 µL of subclone cell culture supernatant and incubate for 2 hours at RT on a rotator (Dynabeads Mixer, obtained from Invitrogen, located in Carlsbad, California).

5. Centrifuge sample, remove supernatant, and resuspend pellet in 1-mL buffer. Repeat.

6. Wash magnetic beads in 2× protein-free T20 blocking buffer prior to use.

7. Resuspend in 1-mL buffer and add 25 µL of paramagnetic beads (equal volumes of Dynal Protein A and Protein G paramagnetic beads; obtained from Invitrogen).

8. Incubate for 30 minutes at RT on rotator.

9. Place tube on magnet (DynaMag-2; obtained from Invitrogen) for 3 minutes.

10. Remove liquid containing unbound veligers.

11. Resuspend pellet containing captured veligers in 1-mL buffer.

12. Enumerate both captured and unbound fractions by light microscopy.

3.6 Scaled-Up Monoclonal Antibody Production and Purification

Candidate subclones for scaled-up mAb production and purification were selected based on the intensity of fluorescence when screened against veligers, as well as ensuring that different patterns of fluorescent staining, multiple parental fusion hybridomas, multiple subclones, and both subclasses of IgG were represented. These subclones were grown as suspension cultures in 150-mL spinner vessels for 3 to 4 weeks, and the antibodies were then purified from cell culture supernatants by Protein G affinity chromatography.

All purified antibodies were diluted to a final concentration of 2 mg/mL and stored frozen in 1-mL aliquots.

4. Results

4.1 Screening Cell Culture Supernatants as Candidates for Final Monoclonal Antibody Production

The 20 mAb-producing hybridoma subclones were used to stain veligers from Lake Havasu using indirect immunofluorescence microscopy. A variety of staining patterns was observed by epifluorescence microscopy, including staining the entire internal veliger contents, or just some regions, within veligers (figure 9). Negative staining controls demonstrated weak autofluorescence (figure 10). Wild veligers from Lake Havasu were used to assess immunofluorescence staining; therefore, there was no control over the life cycle stages that could be tested. However, one of the subclone supernatants (2E10-1D6) stained an apparent quagga trocophore (figure 11).

The 3C4-2F5 subclone supernatant produced a speckled pattern of fluorescence with multiple foci of intense staining within veligers against an overall background of less intense staining (figure 12). For some of the subclones, staining was focused in the vellum at the shell opening (figure 13A-C), while other subclones produced intense staining that was localized to a single internal region of the veliger, which may be a particular organ or part of an organ (figure 13D). Overlaying fluorescence images onto light microscopy images of veligers displayed the extent of internal staining (figure 14).

All of the supernatants with high C:Q ratios (>10), which were due to relatively strong binding to *Corbicula* protein, were the IgG1 subclass (table 1). These subclones are not good candidates for scaled-up antibody production and purification because they are not quagga specific, based on ELISA screening. Interestingly, the fluorescence staining with cell culture supernatants from all of these subclones was restricted to a single, well-defined region of the veligers, indicating that the antibody was recognizing a specific localized protein or tissue; the staining pattern corresponded to a region of the veliger that appeared dark under visible microscopic illumination, and the staining was located on one side of the umbonal region (figure 15).

All of the IgG2 antibody-producing subclones displayed strong staining of most of the internal veliger tissues. Antibodies that generated localized staining, such as around the edge of the shell opening, multiple distinct regions within veligers, or a single distinct region, all belonged to the IgG1 subclass.

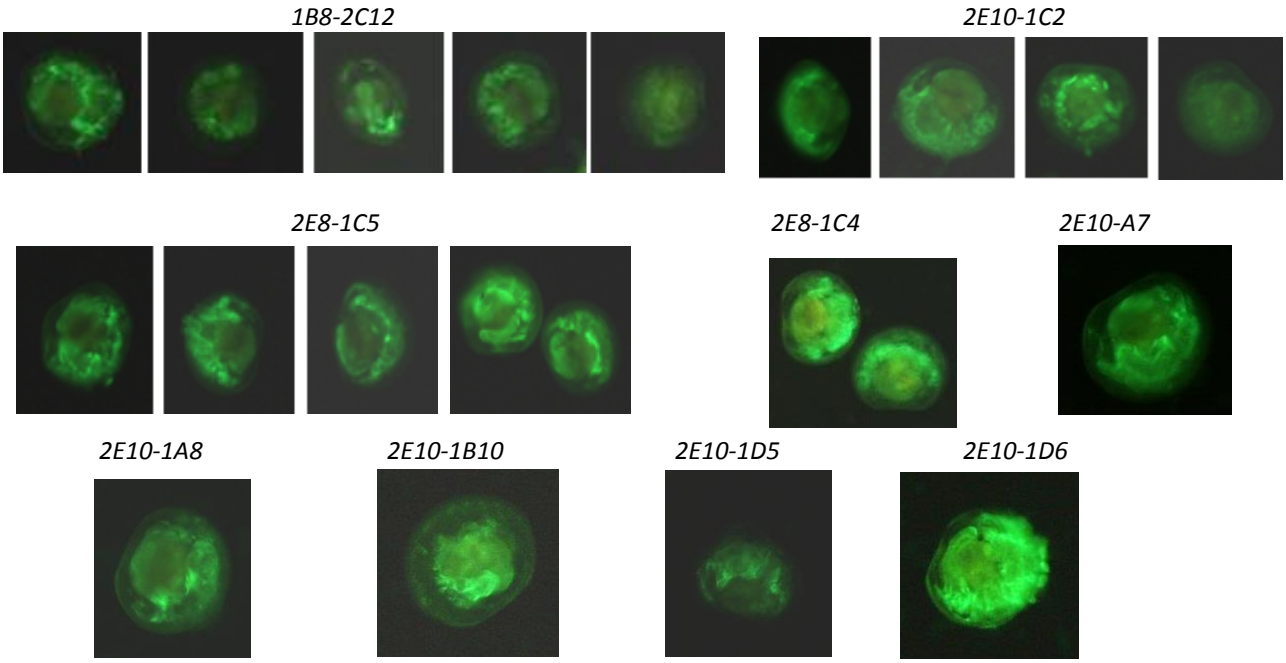

Figure 9. Representative immunofluorescence patterns of veligers stained using cell culture supernatants from the indicated mAb-producing hybridoma subclones. Veligers were stained with 500 µL of hybridoma cell culture supernatant containing IgG monoclonal antibody and an anti-mouse-IgG, FITC-conjugated secondary antibody.

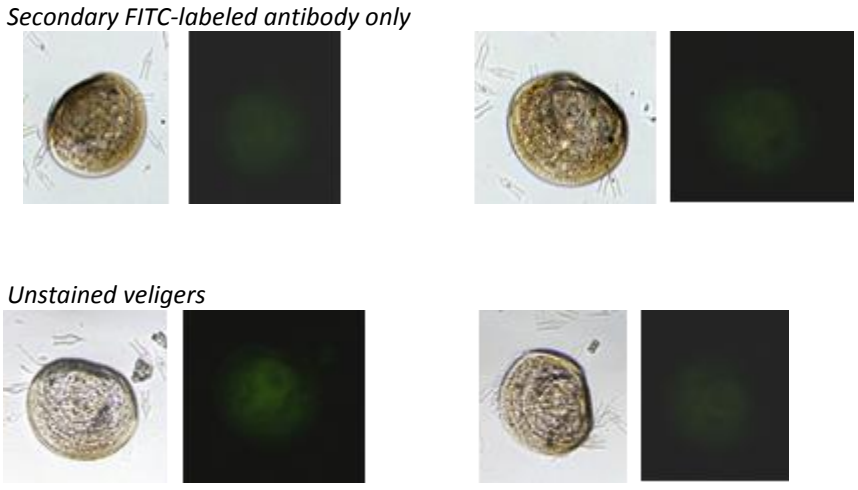

Figure 10. Fluorescence patterns of negative staining controls alongside the corresponding light microscopy images of each veliger.

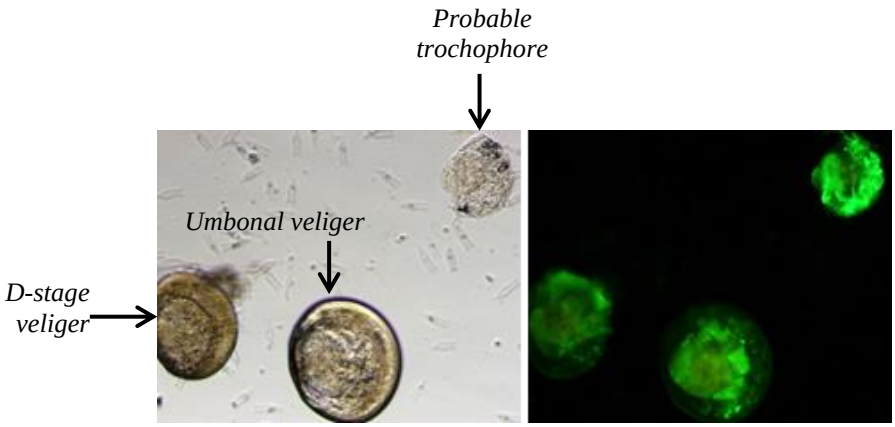

Figure 11. D-shaped and umbonal veligers and a possible trochophore stained with cell culture supernatant from hybridoma subclone 2E10-1D6. Left panel shows white light microscopy, and right panel shows epifluorescence microscopy.

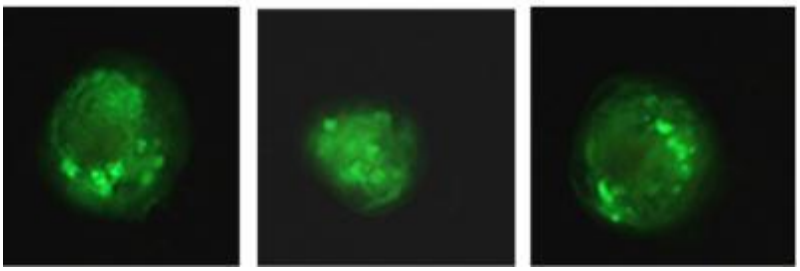

Figure 12. Fluorescence staining pattern for subclone 3C4-2F5 showing multiple discrete foci of intense staining against a background of lighter staining.

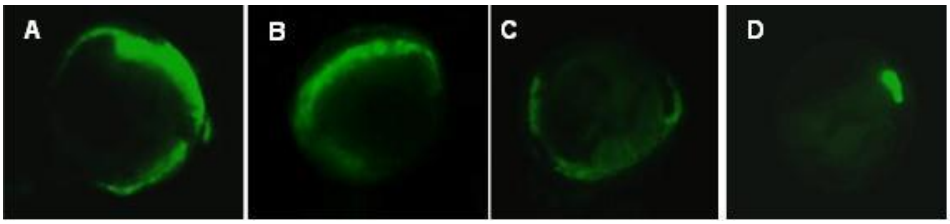

Figure 13. Localized staining of quagga mussel veligers with mAb in hybridoma cell culture supernatants: (A) 3E9-2C10; (B) and (C) 3C4-2B11; and (D) 2E8-1C5.

Figure 14. Fluorescence staining patterns overlaid on light microcsopy images of veligers.

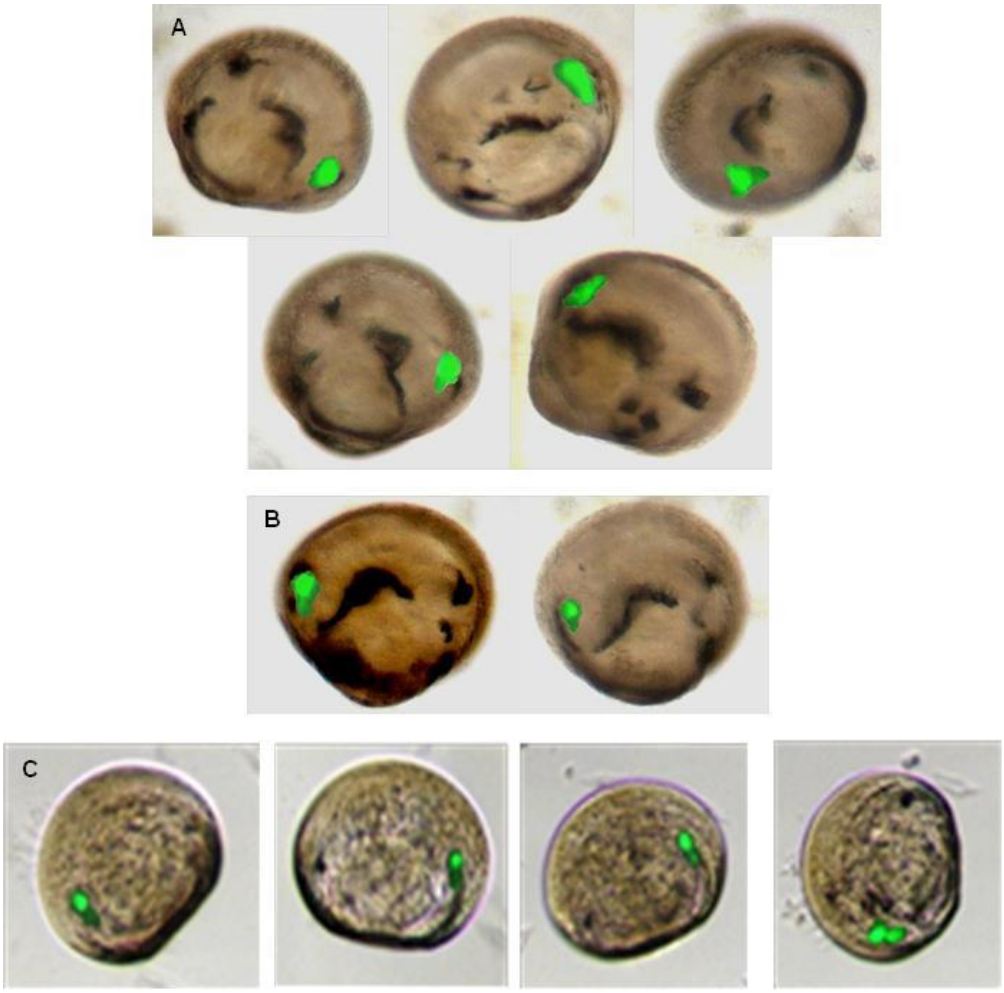

Figure 15. Localized binding of anti-quagga mussel monoclonal antibodies visualized by indirect immunofluorescence microscopy. Veligers were stained with IgG-containing hybridoma cell culture supernatants and an FITC-conjugated, anti-IgG secondary antibody. Cell culture supernatants were from hybridomas 3E9-2C10 (A), 4E12-2H10 (B), and 4E12-1G4 (C). Fluorescence images (green) were overlaid on light microscopy images.

4.2 Assessment of Purified Monoclonal Antibodies by Immunofluorescent Staining

Eight subclones were selected for large-scale (150-mL) antibody production and purification (table 2), representing four parental hybridomas and both types of IgG. Final, post-purification yields varied greatly (~0.05 to 30 mg), but the yield did not correlate with either the parental clone or IgG subclass. Due to the very low yield of 1B8-2C12, new subclones were generated from the parental hybridoma (1B8-2E9, 1B8-1H9, and 1B8-8H9), which were screened against veligers by immunofluorescence microscopy (figures 16 and 17). Subclone 1B8-2E9 was selected for scaled-up mAb production and purification, based on fluorescence intensity and overall staining pattern. Large-scale production and purification were also repeated for the 3C4-2F5 subclones, increasing the yield from 1.6 mg to 6 mg.

Table 2. Subclones Selected for Scaled-Up mAb Production and Purification

Hybridoma Subclone	Isotype/ Subclass	Antibody Yield (mg)
2E10-1A8	IgG2b k	7.2
2E10-1B10	IgG2b k	30
2E10-1E12	IgG2b k	20
2E8-1C5	IgG2b k	Cell line stopped producing antibody
1B8-2C12	IgG2b k	~0.05
1B8-2E9	IgG2b k	13.4
3C4-2B11	IgG1 k	21
3C4-2F5	IgG1 k	1.6 / 6

The 2E8-1C5 hybridoma initially produced antibody, and unpurified antibody in cell culture supernatant stained the internal tissues of veligers (figure 19), so it was selected for scaled-up antibody production. However, during the scale-up process, the cell line stopped producing antibody. Hybridomas are artificial cell lines that can become unstable due to mutations and chromosome loss, potentially affecting antibody yield and quality. If these mutations occur in the genes encoding the heavy or light chains of the IgG molecule, production of active antibody can stop completely (Kromenaker and Srienc, 1994).

As with the polyclonal antibodies and unpurified mAb in cell culture supernatants described above, purified mAbs were assessed by immunofluorescence microscopy and magnetic purification of veligers. Again, a variety of immunofluorescence staining patterns was observed, including just around the periphery of veligers, a speckled or granular pattern of intense areas of staining against an overall weaker background, and staining of the entire internal structure of veligers (figures 16-18).

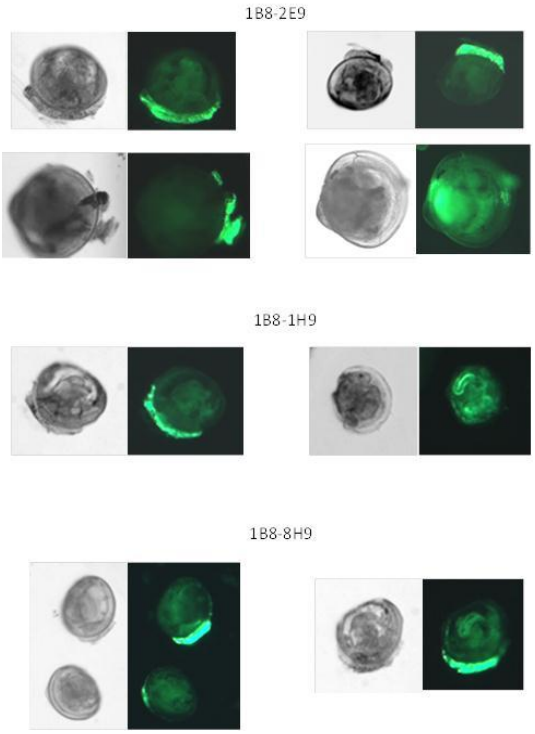

Figure 16. Indirect immunofluorescent staining of Lake Havasu veligers with the recloned 1B8 hybridoma cell culture supernatants. Veligers were fixed in MSE and stained with 500 µL of mAb hybridoma culture supernatant and goat anti-mouse IgG-FITC conjugate.

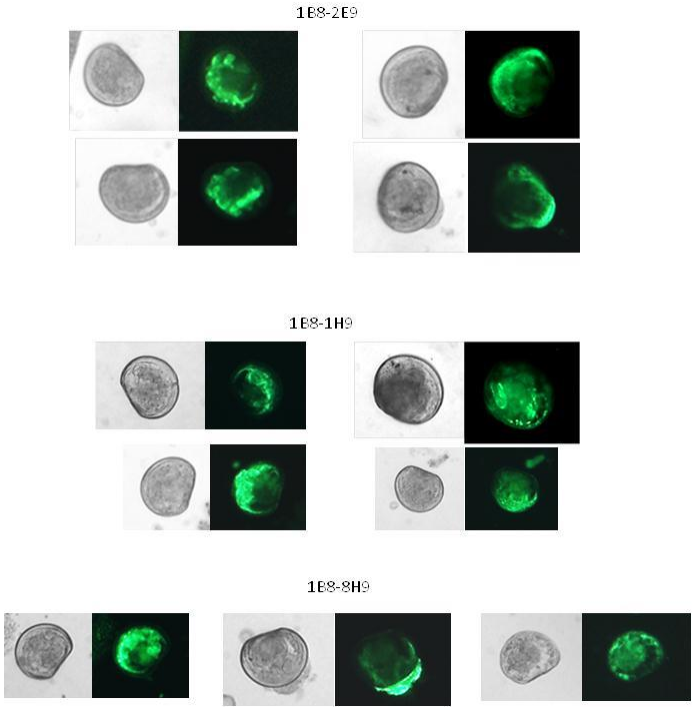

Figure 17. Indirect immunofluorescent staining of Copper Basin veligers with the recloned 1B8 hybridoma cell culture supernatants. Veligers were fixed in MSE and stained with 500 µL of mAb hybridoma culture supernatant and goat anti-mouse IgG-FITC conjugate.

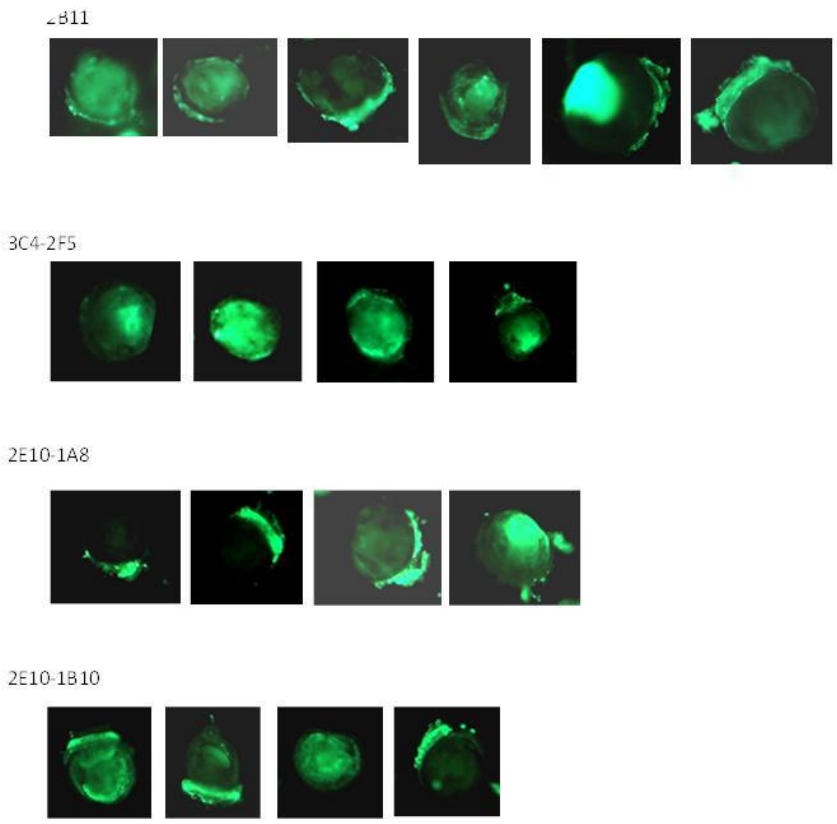

Figure 18. Representative immunofluorescence patterns of veligers stained using the indicated purified monoclonal antibodies. Veligers were stained with 100 µL of mAb and an anti-mouse-IgG, FITC-conjugated secondary antibody.

Strong autofluorescence is common in marine bivalve larvae (Heaney et al., 2011), and multiple sources report autofluorescence as a potential problem when using fluorescence technologies to examine marine bivalves. Autofluorescence is caused by endogenous fluorophores such as lipofuschin, chlorophyll, collagen, elastin, nicotinamide adenine dinucleotide hydride, riboflavins, and flavin coenzymes. Dreissenid mussels contain many of these same types of compound; therefore, autofluorescence is also expected with quagga mussel veligers. In addition, an immunohistochemical study demonstrated that portions of the adult zebra mussel foot tissue were autofluorescent when excited at 488 nm and 568 nm (Anderson and Waite, 2000). Autofluorescence was most intense in regions of the byssal gland and in the secretory products in the ventral groove.

Lipofuschin is localized in digestive gland cells of the marine mussel *M. edulis* (Hole et al., 1993). It is a pigment that naturally accumulates in some types of cells. Lipofuschin content increases with mussel age and in response to certain environmental stressors or pollutants. The lipofuschin content of digestive gland cells in *M. galloprovincialis* also displays seasonal fluctuations (Koukouzika et al., 2009). Consequently, the level of autofluorescence may fluctuate depending on environmental conditions at the time of larval collection.

In the present study, weak autofluorescence was observed when examining negative staining controls using just the secondary antibody or no antibodies at all (figure 10). Most of the immunofluorescent staining was conducted using veligers fixed in 25% ethanol, which is the standard preservative used for MWDSC's routine veliger monitoring program. Heaney et al. (2011) investigated autofluorescence reduction techniques for *M. edulis*. Out of eight chemical fixatives, samples fixed in MSE displayed the lowest autofluorescence. When combined with an Autofluorescence Eliminator Reagent (EMD Millipore) or a saturated solution of Sudan Black in 95% ethanol, autofluorescence was reduced by 60 to 80%. Sudan Black binds to lipofuschin and similar lipid-based materials, thereby blocking their autofluorescence. Consequently, the most recent evaluations of immunofluorescent staining using the monoclonal antibodies in this project were conducted on MSE-fixed veligers treated with Sudan Black to reduce autofluorescence.

4.3 Magnetic Capture Using Purified Monoclonal Antibodies

An important application of an antibody that recognizes quagga mussel veligers will be magnetic immunocapture to separate veligers from other organisms and sample debris, thereby simplifying identification and enumeration of veligers in these "cleaned" samples. Previous experiments using polyclonal antiserum and purified antibodies demonstrated recovery efficiencies of 22 to 100% for nonchlorinated samples, using a variety of secondary "bridges" to link the primary antibody to paramagnetic beads (table 3). Although recovery efficiencies varied widely, most of the results with polyclonal antibodies demonstrated that far more paramagnetic beads bound to veligers when the beads were conjugated to pR1030, compared to beads alone, proving that the pR1030 antibody recognized and bound to veligers. However, in most experiments, some veligers were observed with attached beads in the absence of antibody, indicating some nonspecific binding between veligers and magnetic bead conjugates, or physical entrapment of beads by veligers. Low recovery efficiencies were obtained for veligers from Lake Skinner (mean for all buffers = 10.7%). Lake Skinner is periodically chlorinated to control the veliger population, and this chlorination may partially degrade (oxidize) antibody binding sites on veliger surfaces or tissues, leading to reduced antibody binding efficiency and lower recoveries.

Immunomagnetic capture recovery efficiencies using unpurified mAb from cell culture supernatants or purified mAb were not as high as those achieved with polyclonal antibodies (table 4). The reason for these low recovery efficiencies is not clear but indicates the necessity of further optimizing immunocapture procedures. Many magnetic beads bound to veligers when unpurified mAb in hybridoma culture supernatants was used as the primary antibody (figure 19); however, recovery efficiencies remained low. When purified mAb was used for immunocapture experiments, very few beads attached to veligers (figure 20).

Low recovery efficiencies may be due to poor binding of the Protein A/G-conjugated paramagnetic beads to the primary anti-quagga antibody or insufficient or inappropriate blocking of non-specific binding sites. It is also possible that the antigenic binding sites were inside veligers at locations that were inaccessible to the relatively large (2.8 µm diameter) antibody-coated paramagnetic beads.

Table 3. Immunocapture of Quagga Mussel Veligers Using Polyclonal Antibodies

Sample Location	Antibody	Bead-Antibody Bridge	Recovery Efficiency
Lake Havasu	pR1000	Dynabeads Protein A	34-87%
	R1030 antiserum	Dynabeads Protein A	96%
	pR1030	Dynabeads Protein A and/or Protein G	33-94%
		Proteon Protein A and/or Protein G	43-46%
		Dynabeads M280 anti-rabbit IgG	31-96%
		GeneScript Protein AG	22-38%
		Pierce Protein AG	100%
Copper Basin	pR1030	Proteon Protein A and/or Protein G	34-55%
		Protein A and/or Protein G	19-61%
Lake Skinner	pR1030	Dynabeads Protein A	8-12%*

* This sample location is chlorinated on a routine basis to control mussel populations.

Table 4. Immunomagnetic Capture of Quagga Mussel Veligers Using Monoclonal Antibodies

Antibody	Recovery Efficiency
Unpurified mAb in cell culture supernatants	1.8-19.1%
p3C4-2F5	2.1-8.3%
p3C4-2B11	1.9-12.2%
p2E10-1A8	7-41.2%
p2E10-1B10	5.6-23.8 %

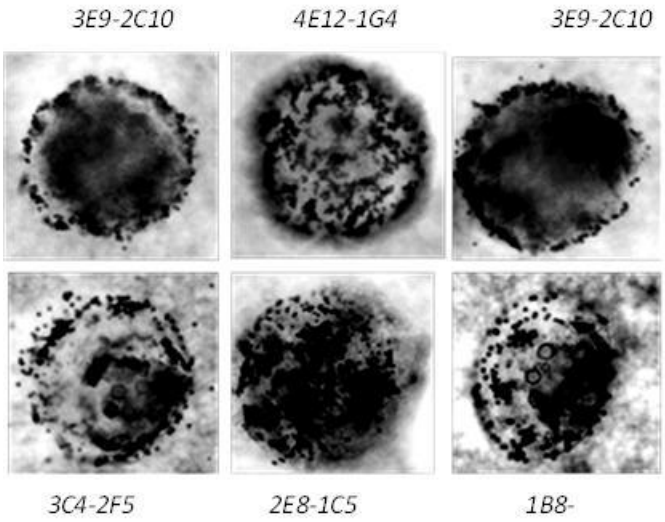

Figure 19. Light microscopic images of paramagnetic beads attached to veligers via unpurified monoclonal antibody in cell culture supernatants from the indicated subclones.

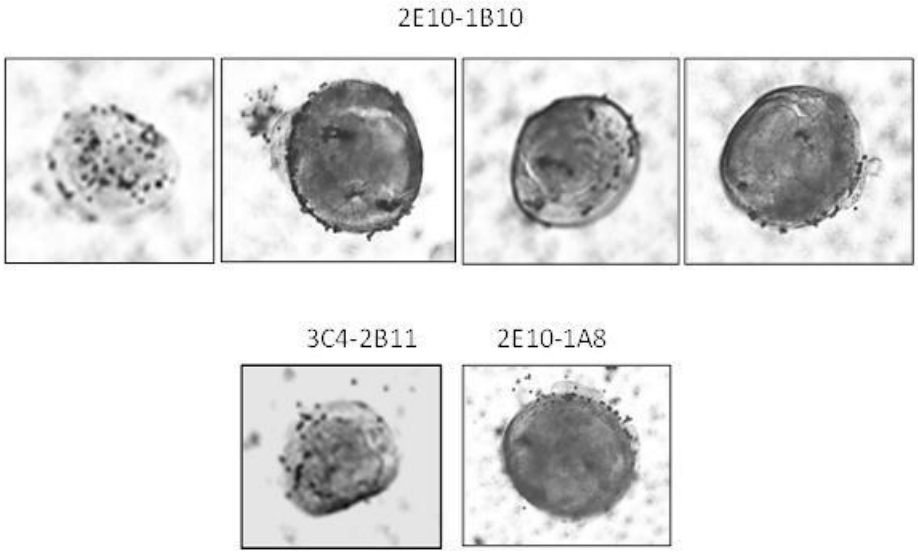

Figure 20. Light microscopic images of paramagnetic beads attached to veligers via purified mAb from the indicated subclones.

5. Discussion

Relatively few studies have investigated the production of antibodies against bivalve mussels or their use for detection and identification of larval stages. The most extensive body of work focused on developing monoclonal antibodies against the marine bivalve *Mytilus galloprovincialis* and their subsequent use for larval detection by immunofluorescence microscopy (Lorenzo-Abalde et al., 2003; Lorenzo-Abalde, 2005; Perez et al., 2009). Monoclonal antibodies were also used to characterize hemocytes in *M. galloprovincialis* using immunofluorescence and immunoperoxidase assays (Carballal et al., 1997). Polyclonal antibodies were also used for detection and identification of *Pecten maximus* (scallop) larvae (Paugam et al., 2000). Immunological techniques using polyclonal antibodies have also been used to investigate structural and physiological characteristics of adult bivalve mussels, including zebra mussels (Sáez et al., 1991; Anderson and Waite, 2000).

Preliminary studies by MWDSC and the Bureau of Reclamation demonstrated the feasibility of generating polyclonal antibodies that recognize quagga mussel veligers. These polyclonal antibodies were used for immunofluorescent staining and magnetic capture of veligers. Polyclonal antibodies are relatively inexpensive and quick to produce. They are stable, tolerant of a wide range of pH and buffer conditions, and generally have high affinity for their target antigen. However, they can display substantial cross-reactivity with nontarget organisms or antigens, and there may be significant variability in antigenic recognition between different batches of the antibody, which have to be produced each time fresh antibody is needed.

The current project built on this previous success by generating monoclonal antibodies that recognized quagga mussel veligers. The advantages of monoclonal over polyclonal antibodies include: greater consistency, higher specificity, and easier production of identical batches of the identical antibody in relatively large quantities. However, they are more expensive and time consuming to produce, compared to polyclonal antibodies, and have more demanding storage conditions.

The project successfully produced seven monoclonal antibodies that generated a variety of immunofluorescent veliger staining patterns. These antibodies bound to veliger tissue and produced three types of staining patterns: (1) staining just around the periphery of veligers; (2) many localized areas of staining within veligers, producing a speckled pattern of intense areas of staining against an overall weaker background; and (3) staining of the entire internal structure of veligers. Variability in staining patterns and staining intensity within veligers may be due to differential degradation of antigens within veliger populations or differential antigen expression induced by physiological conditions during different larval stages. Similar staining patterns, particularly around the edge of larvae, were observed by indirect immunofluorescence using a mAb developed

against *M. galloprovincialis* (Lorenzo-Abalde et al., 2003; 2005); the authors reported antibody-specific staining patterns with an IgG2b mAb producing a granular (speckled) staining pattern and an IgG1 mAb binding to the entire inner area of larvae. These same staining patterns were maintained throughout all stages of larval development. Although we also observed some antibody-specific quagga veliger staining patterns with the very localized staining displayed by 3E9-2C10, 4E12-2H10, and 4E12-1G4 (figure 15), some variation in staining patterns appeared to be more dependent on veliger condition or age, rather than the particular mAb. For example, the three recloned 1B8 hybridomas mostly stained the exposed velum around the periphery of veligers recovered from Lake Havasu (figure 17), whereas the same antibodies produced more internal and granular staining with veligers from Copper Basin (figure 18).

Considering the generally successful trials of immunomagnetic capture using polyclonal antibodies, the poor performance of this technique with the mAbs, particularly the purified mAbs, was disappointing. Low recovery efficiencies may be due to poor binding of the Protein A/G-conjugated paramagnetic beads to the primary anti-quagga antibody or insufficient or inappropriate blocking of nonspecific binding sites. It is also possible that the antigenic binding sites were inside veligers at locations that were inaccessible to the relatively large (2.8 µm), antibody-coated paramagnetic beads.

The Fc region of the IgG mAb was utilized for final antibody purification by Protein A affinity chromatography. It is possible that the Fc region was altered during this process so that subsequent binding of Protein A-linked magnetic beads was impeded. A similar study reported good immunofluorescent staining of bivalve larvae but poor magnetic capture (Paugam et al., 2000). A polyclonal antibody against the bivalve scallop *P. maximus* was used to stain larvae using secondary antibodies conjugated to FITC or alkaline phosphatase. However, although antibody-paramagnetic bead (M280) complexes bound to scallop larvae, they did not "magnetize" them enough to allow magnetic capture.

All of the antibody-producing cell lines developed for this project are stored as frozen (-80 °C) stocks at QED Bioscience, Inc., and at MWDSC's Water Quality Laboratory. These frozen cell stocks can be used to generate additional purified antibody if necessary. Additional work to further the development and refinement of these antibody-based veliger detection tools includes: (1) evaluating staining patterns and intensity with all larval stages of quagga mussels (D-shaped, umbonal, and pediveligers); (2) thoroughly evaluating specificity and quantification of false-positives and false-negatives with a variety of nontarget organisms; (3) improving reduction of autofluorescence; (4) evaluating different-sized paramagnetic beads, including <100 nm beads; (5) assessing alternative secondary bridges linked to magnetic beads to improve magnetic capture with mAbs; and (6) identifying and characterizing the protein antigen(s) recognized by the antibodies, using protein separation by polyacrylamide gel electrophoresis and Western blotting.

6. Conclusions

The goal of this project was to produce a mAb that selectively binds to quagga mussel veligers. A variety of mAbs were produced as unpurified, laboratory-scale preparations and as purified, reagent-grade reagents in milligram quantities. These antibodies stained veligers, generating a variety of fluorescence staining patterns when observed by indirect immunofluorescence microscopy. The project demonstrated the feasibility of generating mAbs that recognize and bind to quagga mussel veligers. Therefore, the project was successful. Additional specificity testing is necessary, along with testing the performance of mAbs against veligers in more complex matrices, and continued development of the immunocapture technique is required. Nevertheless, the antibodies produced by this project provide tools that could simplify microscopic detection and identification of veligers in water samples.

7. References

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