

**MONITORING THE AQUATIC FOOD BASE
IN THE COLORADO RIVER, ARIZONA
DURING JUNE AND OCTOBER 2002**

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NAU Aquatic Food Base Project

ANNUAL REPORT

20 February 2003

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Cooperative Agreement - 02WRAG0028**

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SUMMARY

Chapter 1 Objectives

1) Determine if the June 2002 benthic community biomass and composition estimates varied from June 2001 estimates.

Biomass estimates varied significantly between June 2001 and 2002 estimates with an overall decrease in phyto-benthic biomass. Cladophora biomass increased by 27%, this pattern was probably driven by the Lees Ferry site as there was a decrease at all other sites. Detritus, MAMB and macroinvertebrates biomass estimates were lower in 2002 by 65%, 50%, and 27% respectively. We expected to see an increase in phyto-benthic community because of the low volume discharges being released from GCD. The river was clear, Secchi depth >2m and NTU < 10, through out the study site and for >60 d prior to collections. These are typically the ingredients for a surge in phyto-benthic production prior to the higher discharges from July through September, but that did not occur in Marble and Grand Canyons. These observations indicated that variables other than light (PAR) were influencing the phyto-benthic community, possibly nutrients.

2) Evaluate the GCMRC mandated changes in collection protocols.

Increasing sampling size from six to 18 on cobble bars did not improve our understanding of the phyto-benthic community. We analyzed all sites and did not find a significant difference in biomass between collecting six or 18 samples at each site. The 2002 change in collecting protocol mandated by the GCMRC assumed collecting three times more samples would improve our ability to detect change. Therefore, there should have been a significant difference in biomass estimates but there was not. Power analysis of the 18 samples collected at each site indicated that we would need between 60 and 260 samples for an 80% confidence interval to reduce biomass estimate variance at the 0.05 alpha level. If the GCD AMP and GCMRC want to explore new sampling and analysis designs, such as the bio-assessment approach and random collection site, then that should be a research topic that is released for RFP competition. Also, the GCMRC Science Advisors asked that no changes in sampling design be instituted without overlapping collections.

Chapter 2 Objectives

1) Survey the main stem Colorado River and tributaries for the extent of colonization of the invasive New Zealand Mud Snail.

New Zealand Mudsnaileds were observed in all habitats in the Colorado River. They were most abundant in the macrophytes and least abundant, though present in some sites, in the silt/sand habitat. New Zealand Mudsnaileds were observed in the cobble bars of five of the 18 tributaries sampled. However, they

were not observed past 32 m upstream from the main stem. The NZMS has an operculum, which allows it to seal itself in its shell and has been reported to pass through fish unharmed. These alien invaders are a trophic dead-end and may deteriorate the food base in the Colorado River.

2) Estimate the amount of diatom biomass/energy consumed by the New Zealand Mud Snail

New Zealand Mudsnailed account for 20 - 100% of the macroinvertebrate biomass at six cobble bars in Colorado River. These invasive snails were probably consuming the majority of the available epiphytic diatom assemblage. Size class data indicates that there were many populations of the NZMS. This information is important because it shows that these snails can take advantage of micro-habitat conditions to reproduce and that a single management action is not likely to control these aquatic pests. One female carries 20 embryos and may be responsible for >1 million offspring within one year, under proper conditions. Dispersal is primarily from unintentional recreational transport.

Aquatic food base status and trends

Average biomass and density estimates (\pm se) in the Colorado River from Lees Ferry (rkm 0.8) to 205 Mile Rapid (rkm 328.8) in June from 1991 - 2002 from cobble/riffle habitats. This table depicts the change in primary producer composition from Cladophora to Miscellaneous Algae, Macrophytes, and Bryophytes (MAMB) and a corresponding change in macroinvertebrate density. These data show how unstable this artificial aquatic community has been over the past decade

Year	<u>Cladophora</u> gAFDM/m ²		MAMB gAFDM/m ²		Invertebrates #/m ²		Snails #/m ²	
1991	2.7	(1.0)	0		150	(57)	2	(0.6)
1992	0.7	(0.3)	0.04	(0.01)	191	(72)	4	(1.3)
1993	1.5	(0.6)	0.08	(0.02)	197	(74)	4	(1.1)
1994	5.2	(1.9)	1.5	(0.5)	738	(280)	13	(4.2)
1995	12.0	(4.5)	1.5	(0.6)	427	(162)	6	(1.9)
1996	7.0	(2.6)	15	(5.2)	1160	(440)	58	(19.1)
1997	3.8	(1.4)	6.2	(2.1)	2500	(950)	970	(320)
1998	6.1	(2.3)	6.6	(2.3)	4773	(1813)	3336	(1100)
1999	5.2	(1.8)	8.0	(2.8)	2237	(850)	640	(211)
2000	2.0	(0.7)	38.0	(13.3)	1116	(424)	37350	(12k)
2001	6.2	(2.3)	36.1	(12.6)	995	(391)	2624	(865)
2002	5.8	(2.7)	10.9	(5.2)	1224	(469)	1969	(839)

ACKNOWLEDGMENTS

We thank our NAU field staff for their efforts during collection trips; Alexis Baca-Spry, Marty Schlien, Carrie Link, Rob Trathnigg, Kristen Zinnamon, and Ken Coleman. Charity Yazzie won an essay contest coordinated with the NAU Inter-Tribal Environmental Student internship provided by the Aquatic Food Base Project.

This project would not be successful without the enthusiastic efforts of our volunteers: Suzy Elisson, Claudia Martinez, Casey Ray, Dave Franklin, and Ari Martinez. Pat O'connell from Montana State University assisted with NZMS survey in October.

We are also grateful for the logistical and technical support from Carol "Fritz" Fritzinger, Dr. Barb Ralston, Dr. Michael Yard and Dr. Steve Gloss of the Grand Canyon Monitoring and Research Center. Melanie Caron, Bill Meyer (DOI), and Susan Hueftle from the GCMRC were also very helpful during collection trips. Stephanie Wyse, GCMRC, provided timely assistance with aerial photographs.

INTRODUCTION

Ecologically based flows (eco-flow) from river regulation structures have recently been discussed as a method to minimize the impact on river ecosystems of hydro-power dams (Freeman et al., 2001). Flow regimes based on pre-dam discharge patterns provide the physical habitat that native organisms evolved with and require to maintain healthy populations (Humphries and Lake, 2000). Design of these eco-flows need to consider flow magnitude, frequency, duration, timing, and ramping rates to be comparable with pre-dam flow regimes (Poff et al., 1997). Delineating these hydraulic variables allows decision making stakeholders and researchers to plan experimental eco-flows within the range of natural variability thereby increasing the probability of attaining management goals (Richter et al., 1997; Stanford et al., 1996). Ricciardi and Rasmussen (1999) reported extinction rates for native fish in North America alone were 1000 times greater this century (40 out of 1061 fishes) than the historical background rate. River modification (eg. dams, inter-basin transfers, reservoir storage capacity and evaporation) is the leading cause of fish extinction. The Colorado River basin was defined as the most “strongly affected” river through modification in North America by Dynesius and Nilsson (1994).

In the Colorado River through Grand Canyon, Glen Canyon Dam (GCD) operations have created an artificial cool-clear stenothermic and autochthonous carbon based river dominated by alien taxa (Shannon et al., 2001). This contrived aquatic ecosystem has replaced a thermally variable, turbid, and allochthonous carbon base river that supported eight native fish in Grand Canyon at the turn of the century; today four remain including the endangered humpback chub (*Gila cypha*; Haden et al., 1999). As a result of the effects of GCD, a 1995 environmental impact statement recommended changes in operations and further study on warming the Colorado River through penstock modification and

releasing surface water from Lake Powell (Robinson and Childs, 2001; USDI BOR, 1995).

Justification for long term aquatic food base monitoring:

Grand Canyon National Park Colorado River Management Plan (NPS 1989) states that its resource management goals are "to preserve the natural resources and environmental processes of the Colorado River corridor and the associated riparian and river environments....(and) to protect and preserve the river corridor environment (NPS 1989:9). Among its objectives are: 1) "establish a long-term monitoring program to assess changes in the status of natural...resources. This program will require definition of present resource status (NPS 1989:10)", and 2) "advocate and support operational objectives for the GCD which are most compatible with protection of the intrinsic resources of the Colorado River within Grand Canyon National Park" (NPS 1989:10). The aquatic food base is an integral part of the natural resources in Grand Canyon National Park.

The Environmental Impact Statement (USBR 1995) on the operation of GCD identified the aquatic food base as an "indicator resource" and important habitat for wildlife. Wildlife linked directly to the aquatic food base include native and non-native fish, insectivorous birds and bats, reptiles and waterfowl. Indirect links to the aquatic food base include peregrine falcons feeding on waterfowl, swifts, swallows and bats, as well as king fishers, great blue herons, osprey and bald eagles preying on fish.

This project also provides data supporting the following Grand Canyon Adaptive Management Program Goals, and Management Objectives (MO)

Goal 1. Protect or improve the aquatic food base so that it will support viable populations of desired species at higher trophic levels.

MO 1.1 Maintain or attain primary producer biomass and composition in the Glen Canyon reach.

MO 1.2 Maintain or attain primary benthic invertebrate biomass and composition in the Glen Canyon reach.

MO 1.3 Maintain or attain primary producer biomass and composition in the Colorado Ecosystem below the Paria River.

MO 1.4 Maintain or attain primary benthic invertebrate biomass and composition in the Colorado Ecosystem below the Paria River.

MO 1.5 Maintain or attain drift biomass and composition in the main stem and tributaries.

2002 Annual Report Objectives:

Chapter 1

- 1) Determine if the June 2002 benthic community biomass and composition estimates varied from June 2001 estimates.
- 2) Evaluate the GCMRC mandated changes in collection protocols.

Chapter 2

- 1) Survey the main stem Colorado River and tributaries for the extent of colonization of the invasive New Zealand Mud Snail.
- 2) Estimate the amount of diatom biomass/energy consumed by the New Zealand Mud Snail

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Chapter 1: June 2002 benthic monitoring in the Colorado River below Glen Canyon Dam, Arizona.

INTRODUCTION

The structure of the benthic community in the Colorado River through Grand Canyon has been altered by the construction of Glen Canyon Dam (GCD) through changes in river discharge, organic budget, suspended sediments, and water temperature (Blinn and Cole 1991, Stevens *et al.*, 1997). At present only discharge can be directly managed, which affects the other physical factors. Higher baseflows, reduced peak flow and hourly fluctuation rates are the essential components of the selected discharge criteria from GCD as defined by the environmental impact statement process (Benenati *et al.*, 2000; USBR 1995). A similar reduction in flow regime implemented on the Patuxent River, MD caused a doubling in benthic macroinvertebrate density and improved community condition (Morgan *et al.*, 1991).

Tributary input of suspended sediments also effectively alters the benthic community below the confluence of the Paria River, 28.1 km below GCD and 2.5 km below Lees Ferry, which is designated 0.0 km. The Little Colorado River (98.6 km) also contributes seasonally high loads of suspended sediments. Average annual sediment input from these two tributaries is 8.25×10^6 tonnes, with the Paria River contributing one third of this amount. The Paria River has an average base flow of only $0.77 \text{ m}^3/\text{s}$ (Andrews 1991). This is an atypical example of a second order stream significantly altering the aquatic community of a fourth or fifth order river by reducing water clarity. Annual median discharge from GCD is $345 \text{ m}^3/\text{s}$ (Stanford and Ward 1991); this can dilute the suspended sediments but not without negative consequences to the benthos. The high suspended loads of the Paria and Little Colorado rivers result from the erosion of

soft sedimentary strata common on the arid Colorado Plateau (Beus and Morales 1990).

The purpose of this project is to determine how these two factors, GCD operations and tributary run-off effect the aquatic community structure of the Colorado River through Grand Canyon. Objectives for this report on the aquatic food base include:

- 1) Determine if the June 2002 benthic community biomass and composition estimates varied from June 2001 estimates.
- 2) Evaluate the GCMRC mandated changes in collection protocols.

METHODS

Collection sites were modified by GCMRC/USGS staff in 2002 with two additional sites in Glen Canyon (river kilometer -13.9 and -8.7), while eliminating the Little Colorado Island site (rkm 98.6) located within the humpback chub critical habitat (USBR 1995; Table 1). Sites at Vasey's Paradise (rkm 50.80) and Nankoweap (rkm 80.0) were also added in 2002, these sites had not been sampled since 1997 after that years request for proposals (RFP) competition and approval by a review panel. These sites were eliminated because of sedimentation within the cobble bar which reduced the effectiveness of the Hess; too much sand in the samples and not enough hard substrate. Pool, organic drift and nearshore habitat collections were eliminated in this 2002 protocol change so 18 Hess samples could taken at each site. Although the reason for this change in protocol was not defined in the GCMRC/USGS 2002 Aquatic Food Base RFP competition, it was assumed this was an attempt to reduce benthic sampling variance and monitoring costs.

Modified Hess substrate samplers were used to collect on cobble/bar riffles, 3 transects and 6 samples/transect, in June 2002 (Table 1). Cobble riffle collections were taken at the greatest depth possible or below 142 m³/s which is the minimum flow allowed from GCD. The stage and location of collection was determined by using an abney level, a control point, and the GCMRC/USGS “GUI flow model” (Wiele 1997) that estimated how deep we needed to collect the Hess sample. This protocol “GUI” is unpublished and was developed by Dr. Mike Yard, GCMRC/USGS staff.

Samples were processed live within 48 h and sorted into five biotic categories: *C. glomerata*, *Oscillatoria* spp., detritus, miscellaneous algae and macrophytes, snails and macroinvertebrates. Macroinvertebrates were numerated into *Gammarus lacustris*, chironomid larvae, simuliid larvae, snails, and miscellaneous invertebrates. Miscellaneous invertebrates included lumbriculids, tubificids, trichopterans, terrestrial insects and unidentifiable animals. Detritus was composed of both autochthonous (algal/bryophyte/macrophyte fragments) and allochthonous (tributary upland and riparian vegetation) flotsam. Each biotic category was oven-dried at 60°C and weighed to determine dry weight biomass. Samples were then ashed (500°C, 1 h), and reweighed for ash free dry mass estimates. Six separate Hess samples were collected and preserved in 70% EtOH for taxonomic verification. Three were kept at NAU and three were delivered to GCMRC on 10 October 2002.

We viewed 14 aerial photographs, provided by GCMRC, of the Little Colorado confluence to determine if the collection site, rkm 98.6, could be influenced by turbid water from the LCR while the main stem was clear. These photos were taken from June 1980 through June 2002 under varying flow conditions of both the main stem and LCR.

Analysis

Objective 1) Determine if the June 2002 phyto-benthic community biomass and composition estimates varied from June 2001 estimates.

We used Multiple Analysis of Variance statistical test with categorical biomass estimates (AFDM g/m²) as the response variable and collection trip as the predictor variable. Sites used were the same as 2001 and n=6. All calculations were performed with SYSTAT™ Ver. 5.2 computer software (SYSTAT, Inc., 1992) on ln+1 transformed data.

Objective 2) Evaluate the GCMRC mandated changes in collection protocols.

We used T-test analysis to determine if increasing sampling size from six to 18 on cobble bars provided greater accuracy by comparing the mean AFDM (g/m²) and density (#/m²) estimates from all cobble bars. The first two samples from each transect were coded to indicate six samples, simulating the past 12 years of monitoring, these six were also included in the 18 samples. Analyses were done with SYSTAT™ Ver. 5.2 computer software (SYSTAT, Inc., 1992) on ln+1 transformed data.

Power analysis was used to determine how many samples would be needed to estimate the biomass of our biotic categories at all cobble bars. Alpha was set at 0.05 sigma and delta were determined from the data collected. Analyses were done with JMP IN™ Ver. 5.2 computer software (SAS INST., Inc., 1996).

We compared biomass estimates at three Glen Canyon sites, rkm -13.8 and -6.7 sites were added in 2002, with MANOVA. Analyses was done with SYSTAT™ Ver. 5.2 computer software (SYSTAT, Inc., 1992) on ln+1 transformed data.

Table 1. Collection sites: River Kilometer (rkm), Elevation (m), Orientation, and Reach Type in the Colorado River Below Glen Canyon Dam for cobble-riffle habitats in Glen, Marble, and Grand Canyons, Arizona.

GLEN CANYON				
<u>Name</u>	<u>RKM</u>	<u>Elevation</u>	<u>Orientation</u>	<u>Reach Type</u>
1. -9 Mile Bar	-13.8R	950	Southwest	Narrow
2. -3 Mile Bar	-6.8L	949	Southwest	Narrow
3. Lees Ferry Cobble	0.8R	947	Southwest	Wide
MARBLE CANYON				
<u>Name</u>	<u>RKM</u>	<u>Elevation</u>	<u>Orientation</u>	<u>Reach Type</u>
4. Two-mile Cobble	3.1R	944	South	Wide
5. Vasey's Paradise	50.8C	876	East	Narrow
6. Nankoweap	80.3C	828	South	Wide
GRAND CANYON				
<u>Name</u>	<u>RKM</u>	<u>Elevation</u>	<u>Orientation</u>	<u>Reach Type</u>
7. Tanner Cobble	109.6L	808	Southwest	Wide
8. 127 Mile Rapid	202.9R	616	Northeast	Narrow
9. 205 Mile Rapid	328.8R	427	South	Narrow

RESULTS

Objective 1) Determine if the June 2002 phyto-benthic community biomass and composition estimates varied from June 2001 estimates.

Biomass estimates varied significantly (Table 2) between June 2001 and 2002 estimates with an overall decrease in phyto-benthic biomass (Table 3). Lees Ferry (rkm 0.8) biomass estimates did not vary between 2001 and 2002. Two-Mile Wash (rkm 3.1) biomass varied significantly with detritus and MAMB increasing in 2002 by 71% and 87% respectively. However, macroinvertebrate biomass decreased by 26%. Density estimates indicated that NZMS decreased from 5731 (se \pm 1478) in 2001 to 431 (\pm 126) in 2002. This could be viewed as a positive change of aquatic community health (see Chapter 2) because these invasive snails offer little energy to the river ecosystem. But Gammarus and chironmid larvae also decreased 90% and 35% in 2002 compared to 2001. These two macroinvertebrates are very important to the river ecosystem (Blinn and Cole, 1991; Blinn et al., 1998). At Tanner Cobble (rkm 109.6) Oscillatoria spp. increased 400% while MAMB decreased by 76%. In Middle Granite Gorge (rkm 202.9) Cladophora decreased by two orders of magnitude. Our last site, 205 Mile Rapid (rkm 328.8), Cladophora and Oscillatoria spp. both decreased in biomass. Cladophora biomass dropped by two orders of magnitude while Oscillatoria spp. was reduced by 32%.

Analysis of all sites indicated that the phyto-benthic community did vary significantly between 2001 and 2002 (Table 2). Cladophora biomass increased by 27%, this pattern was probably driven by the Lees Ferry site as there was a decrease at all other sites (Table 3). Detritus, MAMB and macroinvertebrates biomass estimates were lower in 2002 by 65%, 50%, and 27% respectively.

Objective 2) Evaluate the GCMRC mandated changes in collection protocols.

Increasing sampling size from six to 18 on cobble bars did not improve our understanding of the phyto-benthic community (Table 4). We analyzed all sites and did not find a significant difference in biomass between collecting six or 18 samples at each site (Table 4). The 2002 change in collecting protocol mandated by the GCMRC assumed collecting three times more samples would improve our ability to detect change. Therefore there should be a significant difference in biomass estimates but there was not.

Power analysis of the 18 samples collected at each site indicated that we would need between 60 and 260 samples for an 80% confidence interval to reduce biomass estimate variance at the 0.05 alpha level (Table 5). The least significant number (LSN) for Lees Ferry (rkm 0.8) is 91 samples, which would provide a 50% confidence interval or chance of detecting a significant change. At 205 Mile Rapid (rkm 328.8) the LSN is 29 samples and would provide a 36% confidence interval.

We detected significant differences in biomass estimates within Glen Canyon collection sites (Table 6). Detritus, MAMB and NZMS biomass estimates varied between the three Glen Canyon sites, rkm -13.8 -6.7, 0.8, in 2002 (Table 7). Detritus and NZMS biomass estimates varied as much as an order of magnitude. Two orders of magnitude of difference was detected in MAMB biomass estimates between the three sites.

Aerial photos indicated that our LCR Isle (rkm 98.6) collection site is not influenced by run-off from the LCR (Figure 1). Transects for this site extend north into the main stem Colorado. The probability of the LCR flash flooding at a level high enough to cover this island while the main stem is clear and low is remote. Dropping this site in the critical habitat is not a prudent decision by the GCRMC staff.

Table 2. Multivariate (MANOVA) analysis comparing 2001 and 2002 biomass estimates (AFDM g/m²) collected from cobble bars in the Colorado river below Glen Canyon Dam. Only significant univariate response variables are listed (p<0.05). Biotic response variables were Cladophora glomerata (c), detritus (d), Oscillatoria spp.(o), miscellaneous algae, macrophytes and brophytes (m) and macroinvertebrates (b). (n=6/collection)

Source	Wilks' Lambda	df	F-ratio	p	Significant Response Variable
rkm 0.8	0.55	5,6	0.96	0.504	
rkm 3.1	0.12	5,6	11.88	0.003	d,m,b
rkm 109.6	0.14	5,6	7.21	0.016	o,m
rkm 202.9	0.15	5,6	6.63	0.019	c
rkm 328.8	0.07	5,6	16.07	0.002	c,o
all sites	0.75	5,60	3.94	0.004	c,d,m,b

Table 3. Biotic categories that were significantly different between 2001 and 2002 cobble bar biomass estimates. Biotic categories Cladophora glomerata (c), detritus (d), Oscillatoria spp.(o), miscellaneous algae, macrophytes and brophytes (m) and macroinvertebrates (b). (n=6/collection)

Site rkm	Biotic Cat.	2001 AFDM g/m ² (\pm se)	2002 AFDM g/m ² (\pm se)
3.1	d	1.3 (0.5)	4.1 (1.4)
	m	10.8 (6.9)	46.6 (11.0)
	b	5.3 (1.4)	4.0 (0.7)
109.6	o	0.0	4.3 (1.4)
	m	60.1 (37.1)	14.7 (3.4)
202.9	c	2.26 (1.0)	0.02 (0.01)
328.8	c	19.8 (4.5)	0.04 (0.02)
	o	4.12 (0.01)	2.81 (1.1)
All Sites	c	4.1 (1.0)	5.6 (1.4)
	d	5.4 (3.1)	1.9 (0.3)
	m	22.2 (11.1)	11.1 (3.3)
	b	1.9 (0.6)	1.2 (0.2)

Table 4. Sample size comparison for benthic biomass estimates from three cobble bars in Colorado River below Glen Canyon Dam. Independent T-test for samples sizes of n=6 versus n=18. MAMB is the abbreviation for miscellaneous algae, macrophytes and bryophytes. NZMS is the abbreviation for New Zealand mudsnails

Biotic Category		Site rkm		
		0.8 g afdm/m ² (sd)	109.6 g afdm/m ² (sd)	328.8 g afdm/m ² (sd)
<u>Cladophora glomerata</u>	n=18	15.5 (10.5)	0.3 (0.6)	0.04 (0.09)
	n=6	8.1 (7.5)	0.6 (1.0)	0.01 (0.02)
		p=0.08	p=0.45	p=0.26
Detritus	n=18	4.08 (6.1)	0.32 (0.48)	1.3 (2.8)
	n=6	2.5 (4.2)	0.22 (0.43)	0.4 (0.3)
		p=0.51	p=0.63	p=0.18
<u>Oscillatoria spp.</u>	n=18	0	4.3 (5.9)	2.9 (4.6)
	n=6	0	5.3 (6.0)	1.8 (2.8)
			p=0.76	p=0.51
MAMB	n=18	46.6 (46.4)	14.7 (14.5)	3.1 (3.01)
	n=6	21.1 (24.3)	11.3 (15.8)	2.4 (1.6)
		p=0.10	p=0.64	p=0.49
Macroinvertebrates	n=18	2.4 (1.9)	0.18 (0.47)	0.01 (0.02)
	n=6	2.6 (2.1)	0.06 (0.08)	0.004 (0.009)
		p=0.84	p=0.31	p=0.28
NZMS	n=18	0.04 (0.05)	0.009 (0.01)	0.001 (0.002)
	n=6	0.035 (0.05)	0.007 (0.01)	0.001 (0.001)
		p=0.70	p=0.74	p=0.85

Table 5. Results of power analysis to determine cobble bar sample size at Lees Ferry (rkm 0.8) and 205 Mile Rapid (rkm 328.8) in the Colorado River, Grand Canyon from June 2002 collection. Analyses were conducted on macroinvertebrate (AFDM g/m²) biomass estimates (n=18). Least significant number (LSN).

Site	Sigma	Delta	Number	Power	LSN
0.8 rkm	3.13	1.25	18	0.13	91
			38	0.26	
			58	0.39	
			78	0.51	
			98	0.61	
			118	0.70	
			138	0.78	
			158	0.87	
			198	0.91	
328.8 rkm	0.09	0.02	18	0.36	29
			38	0.71	
			58	0.89	
			78	0.96	

Table 6. Multivariate (MANOVA) analysis comparing June 2002 biomass estimates (AFDM g/m²) collected from three cobble bars in Glen Canyon, Colorado River below Glen Canyon Dam. Only significant univariate response variables are listed (p<0.05). Biotic response variables were Cladophora glomerata (c), detritus (d), Oscillatoria spp.(o), miscellaneous algae, macrophytes and brophytes (m), macroinvertebrates (b) and New Zealand Mud Snails (n=18/collection)

Source	Wilks' Lambda	df	F-ratio	p	Significant Response Variable
Site	0.28	10,90	7.98	p<0.001	d,m,n

Table 7. Biotic categories that were significantly different from three Glen Canyon cobble bar biomass estimates, June 2002. Biotic categories Cladophora glomerata (c), detritus (d), Oscillatoria spp.(o), miscellaneous algae, macrophytes and brophytes (m) and macroinvertebrates (b) and New Zealand Mud Snails (n). (n=18/collection)

Biotic Category	Collection Sites					
	-13.8 rkm		-6.7 rkm		0.8 rkm	
	AFDM g/m ² (\pm se)		AFDM g/m ² (\pm se)		AFDM g/m ² (\pm se)	
d	2.1	(0.5)	0.2	(0.07)	4.1	(1.4)
m	1.6	(0.8)	0.07	(0.2)	46.6	(10.9)
n	2.9	(0.9)	4.3	(0.6)	1.4	(0.4)

Figure 1. Aerial photographic of the confluence of the Little Colorado River and main stem Colorado River, Grand Canyon. Collection site transects are indicated by XXX and transect numbers.

DISCUSSION

Objective 1) Determine if the June 2002 phyto-benthic community biomass and composition estimates varied from June 2001 estimates.

In June 2002 we expected to see an increase in phyto-benthic community because of the low volume discharges being released from GCD. Flows ranged between 227 and 400 m³/s allowing collections below the minimum flow stage of 142 m³/s. Also, the Colorado Plateau is experiencing a drought therefore tributary input of suspended sediments was negligible. The river was clear, Secchi depth >2m and NTU < 10, through out the study site and for >60 d prior to collections. These are typically the ingredients for a surge in phyto-benthic production prior to the higher discharges from July through September, but that did not occur in Marble and Grand Canyons. There observations indicated that variables other than light (PAR) were influencing the phyto-benthic community, possibly nutrients. While on a May private river trip Marty Schlien, a veteran Food Base Project staff member, returned and described large quantities of drifting algae, algae covered rocks and abundant midge hatches through out the river corridor. During June collections we noted very few drifting packets of algae. The only habitats where we saw abundant growth of algae was on cobble bars was just below tributaries and in pools where Potamogetan pectinatus was very prolific. These rooted aquatic macrophytes can derive nutrients from the fine sediments that collect around them in the low velocity areas they create (Benenati et al., 1999) This gives observational evidence that where nutrients occurred so did primary production (within sediments and within a short distance from tributaries).

This June 2002 monitoring data demonstrates that the phyto-benthic community is responding to physical factors other than light (Blinn et al., 1998), and flow (Benenati et al., 2000; Shannon et al., 2000). These most recent changes

in sampling design as required by GCMRC prevented us from documenting these observations because we lacked equipment; dredges for pools, drift nets and nutrient collection supplies. These items clearly demonstrate the limitations of this overly simplistic sampling design. With luck, the GCMRC Integrated Water Quality Program will be able to determine if there are any large scale changes in the nutrient regime within the study site due to Lake Powell draw-down or other factors.

Objective 2) Evaluate the GCMRC mandated changes in collection protocols.

There is no statistical evidence that increasing sample size from six to 18 has improved our ability to detect change in the phyto-benthic community of the Colorado River below GCD. It is well understood in the benthological community that variance is high in streams (Palmer *et al.*, 1997) and that variance can not be “sampled away” as is done in terrestrial monitoring (Kimberling *et al.*, 2001). Conversely, sampling effort can have a statistically significant impact on species assemblage patterns within and between sites based on density estimates (Cao, *et al.*, 2002). However, the NAU Aquatic Food Base monitoring program is a bio-energetics model (Blinn *et al.*, 1994) and not a bio-assessment model (Vinson *et al.*, 2001). We surveyed the stream monitoring literature and could not find a sustained monitoring program with n=18 for a sampling regime (Table 8). The worlds premier lotic organization, North American Benthological Society, queried their membership on monitoring protocol and the majority collected 3 or less at each site (Carter and Resh, 2001). This comprehensive listing of sampling protocol showed that the Colorado River through Grand Canyon monitoring program devised by NAU in 1990, reviewed in 1997 and 2001, with over 22 journal articles and several book chapters is more extensive than most because it

monitors several habitats. This multiple habitat approach is important because of the varying flow regimes and turbidity common in the study site.

In the 2001 Aquatic Protocol Evaluation Program Evaluation (Anders et al., 2001) there was no mention of a need to change sampling design. Furthermore, the 2002 RFP released by GCMRC for the aquatic food base did not state why the change in sampling design nor listed any citations other than a USGS web site on power analysis that can only be accessed by government personnel. Three reviews were obtained by the GCMRC and each one stated that the increase in sampling numbers was not substantiated. For some reason unknown to these authors and the benthic monitoring community at large GCMRC insisted on going ahead with these changes.

Typically monitoring programs increase sampling locations within a study site and not sample numbers to reduce variability (Loab and Spacie, 1994). Also, sites are more commonly randomly selected to reduce sampling design bias, such as within the US Environmental Protection Agencies (EPA) Environmental Monitoring and Assessment Program (EMAP; Herlihy et al., 2000). However, neither Glen Canyon Adaptive Management Program nor the GCMRC/USGS have developed a metric to assess trends over a several year period with the study site. Urquhart et al., (1998) made the case for a sampling and analysis design for the EPA EMAP data on regional lakes. Assumptions made by Urquhart et al., (1998) are not entirely applicable to the Colorado River below GCD benthic community or the GCD AMP. 1) Stable environment over several years for lakes. Recent and ongoing micro-management of GCD flows versus the need to produce results quickly. 2) Trends can be detected over a several year period. The GCD AMP and GCMRC wants to know status and trends at least on a yearly basis. 3) Regional data analysis is applicable to single river data analysis. The GCD AMP is responsible for about 500km of the Colorado River and regional

comparisons are not mandated. The GCD AMP and GCMRC want to know if an experimental flow or the EIS ROD flows had the desired results on the resources they have determined to be important in the ecosystem. Therefore a sampling design needs to determine that in a short time period. The NAU Aquatic Food Base Program has done so repeatedly over the past 12 years (Blinn et al., 1998, Benenati et al., 2000, Shannon et al., 2001).

If the GCD AMP and GCMRC want to explore new sampling and analysis designs, such as the bio-assessment approach and random collection site (Anders et al., 2001), then that should be a research topic that is released for competitive RFP process and not as an add-on to dilute an existing program. NAU Aquatic Food Base Program has the longest sustained biology monitoring program in existence in the GCMRC program. Changes in sampling design should not be taken lightly or interfere with existing protocols if their value is questionable. Given that three reviewers selected by GCMRC did not agree with the sampling changes this should have been an indication to the GCMRC staff that an error was made. Also the GCMRC Science Advisors (April 2002) asked that no changes in sampling design be instituted without overlapping collections.

Table 8. Sample collecting schemes from other established stream/river monitoring programs.

<u>Sample Number</u>	<u>Seasonal Collection</u>	<u>River</u>	<u>Habitat</u>	<u>Authors/Year</u>
6	1x year	6 Restored Rivers in Finland	Cobble/ Moss	T. Muotka <u>et al.</u> , 2000
1	1x year	5 Streams in California	Cobble	C.P. Hawkins <u>et al.</u> , 2000
15	1x year	Terrestrial samples next to Columbia River	Terrestrial	D.N. Kimberling <u>et al.</u> 2001
5	2x year	Mgeni and Darvill Rivers, South Africa	Cobble/ Gravel/Silt	C.W.S. Dickens and P.M. Graham/1998
1	3x year	41(RIVPAC) Rivers in Europe	Cobble/silt/ macrophytes	R.T. Clarke <u>et al.</u> , 2002
1	2x year	11 Rivers Otago Region, New Zealand	Cobble/Silt	B.S. Caruso/ 2002
5	2x year	River Kennet, England	Gravel/ Macrophytes	J.F. Wright <u>et al.</u> 2002
3	1x year	Upper Mississippi River	Silt/Sand	L.A. Bartsch <u>et al.</u> 1998
5	1x year	Columbia River Basin	Cobble/ Silt/Sand	T.F. Cuffney <u>et al.</u> 2000
1-8	1-20x year	Green River, Wyoming	Cobble	M.R. Vinson <u>et al.</u> 2001
4-8	1x year	Clinch, Powell, and Sequatchie Rivers, tributaries to Tennessee Ri.	Cobble/Silt	B.L. Kerans <u>et al.</u> 1992
3	5x year	8 Rivers in Frasier River catchment, Canada	Cobble/ Gravel	P.F. Reece <u>et al.</u> 2001
3	4x year	Cache la Poudre River, Colorado	Cobble/Sand Gravel/Silt	N.J. Voelz <u>et al.</u> 2000

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Chapter 2: Distribution of the invasive New Zealand Mud Snail in the Colorado River and tributaries below Glen Canyon Dam, Arizona.

INTRODUCTION

The exotic New Zealand Mudsnailed, Potamopyrgus antipodarum (Gastropoda: Hydrobiidae, NZMS), has invaded many large drainages and tributaries in the western United States, including the Middle Snake River, Idaho; several streams in the Upper Madison River in Yellowstone National Park; the Green River; and has rapidly become the dominate macroinvertebrate in these communities (Richards et al., 2001). It is listed as an aquatic pest species by the U.S. Fish and Wildlife Service in accordance with the Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990 (P.L. 101-646). In New Zealand, this snail increases densities in degraded habitats and can be considered an indicator species of poor habitats (Towns 1981). Therefore, NZMS invasion has become a concern for managers, yet the effects this species on river ecosystems in the U.S. is unknown.

In 1995 the NZMS established in the Colorado River through Grand Canyon. These tiny snails colonize rapidly through asexual reproduction, with one carrying 20 embryos, so within one year each adult can produce >1 million offspring if conditions are favorable. Previous to NZMS introduction chironomid larvae and Gammarus lacustris dominated the macroinvertebrate community (Blinn et al., 1994; Stevens et al., 1997). These animals provided a critical intermediate link between primary producers and fish in the Colorado River. The NZMS has an operculum, which allows it to seal itself in its shell and has been reported to pass through fish unharmed (Bondesen and Kaiser 1949, Haynes et al., 1985). These alien invaders are a trophic dead-end and may deteriorate the food base in the Colorado River. This threatens native fish fauna,

including the dwindling Humpback Chub population, a trophy trout fishery, and a diverse insectivore fauna.

Objectives for this study were:

- 1) Survey the main stem Colorado River and tributaries for the extent of colonization of the invasive New Zealand Mudsnail.
- 2) Estimate the amount of diatom biomass/energy consumed by the New Zealand Mudsnail and determine if there are any potential trophic interactions.

METHODS

Objective 1) Survey the main stem Colorado River and tributaries for the extent of colonization of the invasive New Zealand Mudsnail.

New Zealand Mudsnail physid, and lymnaeid (three common snails in the Colorado River) densities were estimated in four habitats (when present) at 21 sites on the main stem Colorado River through Grand Canyon from 29 September- 24 October, 2002. The four habitats, included: 1) cobble/boulder, 2) silt/sand, 3) filamentous algae and aquatic vascular plants, and 4) varial zone pools. Habitat collection sites were randomly selected within two meters of shore and three meters in depth. Circular templates (44.2 cm² or 314.2 cm²) were used to quantify samples from cobble/boulder, silt/sand, and filamentous algae/ aquatic vascular plants. Templates were placed on the substrate to mark the area in which the macroinvertebrates were removed, identified, and quantified. Silt/sand habitats were also sampled using a sieve (348 cm²) to scrape sediment 5 cm deep for a length of 20 cm. Macrophytes were collected using a Petite Ponar (232 cm²). Habitats were sampled below the minimum flow (142 m³/s) with discharges ranging from 226 - 350 m³/s.

Eighteen tributaries were sampled near their confluence with the Colorado River and upstream until no snails were observed. Three replicates were collected at each habitat type in a similar fashion as the main stem. The Paria River samples were collected during a small flash flood ($\sim 0.28 \text{ m}^3/\text{s}$), all other tributaries were at baseflow.

Snail voucher specimens were collected and preserved in 70% ethyl alcohol. At each collection site, water velocity (m/s) and water depth (m) was measured. Global positioning satellites (GPS) coordinates were recorded. Water quality characteristics; dissolved oxygen (ppt), specific conductivity (mgL^{-1}), and temperature ($^{\circ}\text{C}$) were measured for each site using a Hydrolab Scout II.

New Zealand Mudsail size class distribution was determined for five sites on the main stem of the Colorado River from 8-17 June, 2002. These data will determine if there is one (similar size class distribution across sites) or several NZMS populations (variable size class distribution across sites). Snails were randomly hand collected from varial zone pools and cobble bars. Collected samples were preserved with 70% alcohol. Snail height was measured from the shell apex to the bottom of the aperture (mm) using a caliper. There were 10 size classes in increments of 0.5 mm from 0.5 to 5.0 mm.

Objective 2) Estimate the amount of diatom biomass/energy consumed by the New Zealand Mudsail and determine if there are any potential trophic interactions.

Eighteen Hess samples were collected from nine long term cobble bar monitoring sites (Table 1, Chapter 1). Samples were processed live within 48 h and sorted into five biotic categories: C. glomerata, Oscillatoria spp., detritus, miscellaneous algae and macrophytes, snails and macroinvertebrates, which were numerated

into Gammarus lacustris, chironomid larvae, simuliid larvae, snails, and miscellaneous invertebrates. Miscellaneous invertebrates included lumbriculids, tubificids, trichopterans, terrestrial insects and unidentifiable animals. Detritus was composed of both autochthonous (algal/bryophyte/macrophyte fragments) and allochthonous (tributary upland and riparian vegetation) flotsam. Each biotic category was oven-dried at 60°C and weighed to determine dry weight biomass. Samples were then ashed (500°C, 1 h), and reweighed for ash free dry mass estimates.

We estimated the biomass of epiphytic diatoms on algal covered cobbles at seven long term monitoring sites (Table 1, Chapter 1). A 20 cm² algal patch was scraped with a razor blade from each cobble, placed on ice, and processed within 48 hr of collection. The phytobenthos was sorted into the categories of Cladophora glomerata, Oscillatoria spp., and miscellaneous algae, macrophytes, and bryophytes. Cladophora, which was selected for the purpose of obtaining epiphyton (diatom) biomass was placed in a Whirl-pak™ containing 100 ml of filtered (0.45 μ m) Colorado River water and shaken for 60 sec to remove epiphyton. This procedure removed at least 80% of diatoms, based on microscopic analysis, and was found to be the most effective technique for separating intact epiphytic diatoms from Cladophora filaments (Blinn et al., 1995). The periphyton suspension was filtered onto Whatman (GF/C) 4.7 μ m glass microfiber filters. All categories were oven-dried for 48 hrs (60°C), and ignited at 500°C for 1 hr to obtain ash-free dry mass (AFDM/m²).

Analysis

Step-wise multiple regression analysis was used to determine if there was a predictable relationship between New Zealand Mudsnail (NZMS), primary producer biomass and other macroinvertebrates density in the Colorado River

below Glen Canyon Dam in June 2002. Analyses was done with SYSTAT™ Ver. 5.2 computer software (SYSTAT, Inc., 1992) on ln+1 transformed data.

RESULTS

Objective 1) Survey the main stem Colorado River and tributaries for the extent of colonization of the invasive New Zealand Mudsnaail.

New Zealand Mudsnaails were observed in all habitats in the Colorado River. They were most abundant in the macrophytes and least abundant, though present in some sites, in the silt/sand habitat (Table 1). New Zealand Mudsnaails were consistently collected from cobble bars. When water velocity was low, NZMS were observed on the surface of cobbles and silt/sand habitats, but in high water velocity they were seen on the underside of cobbles and boulders and/or in an eddy created by boulders or sand rifts. They were collected in varial zone pools, which may provide a refuge during fluctuating flows. Physid snails were not common in the main stem and were found in areas with low water velocity (Table 1). Lymnaeid snails were not observed in the main stem.

Average main stem water quality characteristics were: dissolved oxygen $10.3 \text{ se} \pm 0.14 \text{ mg/L}$; specific conductivity $0.8168 \pm 0.0072 \text{ ms}$; and temperature $11.5 \pm 0.19 \text{ }^\circ\text{C}$. Snails were collected at depths of 0.03 - 2.25 m and in water velocities of 0.0 - 0.57 m/s.

New Zealand Mudsnaails were observed in the cobble bars of five of the 18 tributaries sampled (Table 2). However, they were not observed past 32 m upstream from the main stem. Crystal Creek had NZMS within three meters from the main stem, but snails were not observed 59 m up the tributary. Also, NZMS were observed in Royal Arch Creek within 32 m of the main stem, but were not observed above 110 m up the tributary. In 127-Mile Creek NZMS were observed within 13 m up from the main stem, however, a three meter high

vertical bedrock wall 40 m upstream may have prevented snails from migrating further, since only physid snails were observed 43.2 m upstream. NZMS were observed 0.5 m up Spring Canyon at low discharge, which may be influenced by the main stem. Physid snails were present in 9 of the 18 tributaries (Table 2). In three of the five tributaries with NZMS presence, physid snails were not observed, and when they co-occurred there were two-fold more NZMS than physid snails. Lymnaeid snails were not observed in tributaries.

Average water quality characteristics of the tributaries were: dissolved oxygen 9.14 ± 0.10 mg/L; specific conductivity= 1.5962 (se ± 0.1599) ms, and temperature 17.8 ± 0.24 °C. Snails were collected between depths of 0.02- 0.38 m and in water velocities between 0.01- 0.78 ms⁻¹.

No patterns in size class data were revealed for the five habitats sampled (Table. 3). This indicates that there is a lot of variability in NZMS life history on the Colorado River and therefore multiple populations. For example, 11% of the NZMS small size class ($\leq 1-1.5$ mm) was observed in varial zone pools at Vasey's Paradise (rkm 50.8) , which indicates that reproduction recently occurred. However, no $\leq 1-1.5$ mm NZMS small size class in the adjacent Vasey's Paradise cobble bar. These data indicate that the snails did not reproduce at the same time as the snails in the varial zone pools.

Table 1. Average density of New Zealand Mudsnails (NZMS) and physid snails in the Colorado River below Glen Canyon Dam in October 2002.

River Kilometer	Habitat	NZMS Density (#/m ²)	(±se)	physid Density (#/m ²)	(±se)
-23.2	Cobble	8826	(3849)	0	(0)
-23.2	Macrophytes	11	(0)	0	(0)
0.0	Macrophytes	31104	(17098)	58	(58)
0.0	Cobble	830	(399)	0	(0)
1.0	Cobble	1174	(559)	0	(0)
14.4	Cobble	2461	(1826)	0	(0)
14.4	Silt/Sand	287	(0)	0	(0)
34.6	Cobble	32	(18)	0	(0)
53.6	Silt/Sand	795	(377)	0	(0)
53.6	Cobble	1134	(744)	0	(0)
53.6	Varial Zone Pool	297	(59)	0	(0)
69.1	Cobble	4463	(748)	0	(0)
69.1	Silt/Sand	3065	(1430)	0	(0)
84.7	Cobble	1007	(92)	0	(0)
84.7	Silt/Sand	1034	(329)	0	(0)
84.7	Silt/Sand	16744	(2134)	0	(0)
105.0	Cobble	0	(0)	0	(0)
105.2	Cobble	403	(166)	0	(0)
114.0	Cobble	339	(261)	0	(0)
114.0	Silt/Sand	2012	(2012)	0	(0)
140.7	Cobble	339	(125)	0	(0)
140.7	Silt/Sand	0	(0)	0	(0)
157.1	Cobble	74	(21)	0	(0)
157.1	Silt/Sand	0	(0)	0	(0)
174.1	Cobble	117	(74)	0	(0)
174.1	Silt/Sand	0	(0)	0	(0)
187.1	Cobble	0	(0)	0	(0)
214.9	Cobble	3134	(1752)	8	(8)
214.9	Silt/Sand	0	(0)	0	(0)
217.5	Cobble	191	(73)	0	(0)
217.5	Silt/Sand	0	(0)	0	(0)
231.4	Cobble	191	(120)	0	(0)
231.4	Silt/Sand	0	(0)	0	(0)
236.8	Cobble	135	(79)	0	(0)
236.8	Silt/Sand	0	(0)	0	(0)
345.0	Silt/Sand	0	(0)	0	(0)
360.0	Cobble	212	(111)	0	(0)

Table 2. Average density of New Zealand Mudsnails (NZMS) and physid snails in 18 tributaries of Colorado River below Glen Canyon Dam in October 2002.

Tributary	Habitat	Meters up Tributary	NZMS Density (#m ²) (±SE)		Physid Density (#m ²) (±SE)	
Paria River	Cobble	733.0	0	(0)	0	(0)
Nankoweap Creek	Cobble	600.0	0	(0)	0	(0)
Little Colorado River	Silt/Sand	314.0	0	(0)	0	(0)
Little Colorado River	Cobble	314.0	0	(0)	0	(0)
Lava Chuar	Cobble	380.0	0	(0)	0	(0)
Clear Creek	Cobble	5.0	0	(0)	0	(0)
Bright Angel Creek	Cobble	3.5	0	(0)	97	(65)
Pipe Creek	Cobble	8.0	0	(0)	0	(0)
Crystal Creek	Cobble	3.0	151	(75)	0	(0)
Shinamu Creek	Cobble	103.0	0	(0)	0	(0)
Royal Arch Creek	Cobble	16.0	237	(221)	86	(71)
Royal Arch Creek	Cobble	32.0	4842	(2512)	0	(0)
Royal Arch Creek	Cobble	59.0	0	(0)	0	(0)
Royal Arch Creek	Cobble	110.0	0	(0)	0	(0)
127-Mile Creek	Cobble	8.0	377	(151)	0	(0)
127-Mile Creek	Cobble	13.0	302	(75)	0	(0)
127-Mile Creek	Cobble	43.2	0	(0)	302	(75)
Tapeats Creek	Cobble	8.0	0	(0)	388	(190)
Deer Creek Falls	Cobble	120.0	0	(0)	0	(0)
Kanab Creek	Cobble	20.0	151	(75)	0	(0)
Kanab Creek	Cobble	205.0	0	(0)	75	(75)
Matkatameba Creek	Cobble	50.0	0	(0)	75	(75)
Havasü Creek	Cobble	15.0	0	(0)	38	(38)
Spring Canyon	Cobble	0.5	226	(0)	0	(0)
Spring Canyon	Cobble	58.0	0	(0)	302	(75)
Spring Canyon	Silt/Sand	93.0	0	(0)	297	(28)
Springs Canyon	Cobble	1.2	0	(0)	226	(3)

Table 3. Percent size class distribution of New Zealand Mudsnaills from four cobble bars in the Colorado River through Grand Canyon. River Kilometer (rkm) 50.81 is a varial zone pool. Size classes are in 0.5 mm increments with size class one <1.5mm and nine >5.1.

rkm	Size Class									Total Snails
	1	2	3	4	5	6	7	8	9	
50.81	15	20	11	11	11	8	15	11	3	72
50.8	0	1	3	8	6	15	49	11	6	96
84.7	0	5	12	10	20	27	23	3	0	100
109.6	13	10	9	10	8	23	23	4	0	100
328.8	10	9	10	10	7	7	29	13	3	68

Objective 2) Estimate the amount of diatom biomass/energy consumed by the New Zealand Mudsnail and determine if there are any potential trophic interactions.

There was a significant positive relationship between NZMS, Cladophora and detritus biomass estimates (Table 4.) We also detected significant positive relationship between NZMS and Gammarus biomass estimates. Gammarus biomass is negatively correlated with Oscillatoria spp., but are positively correlated with Cladophora and detritus biomass estimates. These data indicate that NZMS and Gammarus may be competing for the same niche in this reach of the Colorado River. We have documented that Oscillatoria is a poor substrate for Gammarus and chironomids in the study site, primarily because it is a weak host for epiphytic diatoms (Benenati et al., 1998).

New Zealand Mudsnails account for 20 - 100% of the macroinvertebrate biomass at six cobble bars in Colorado River (Table 5). These invasive snails could be consuming the majority of the available epiphytic diatom assemblage (Table 5). Snails in the Colorado River are grazers and consume epiphytic diatoms (Blinn et al., 1995), as do Gammarus and chironomids. Lees Ferry (rkm 0.8) epiphytic diatom biomass estimates are an order of magnitude lower than what Benenati et al., (1998) reported in 1992 prior to the colonization of these snails.

Table 4. Results of step-wise multiple regression analysis to determine if there is a predictable relationship between New Zealand Mudsnail (NZMS), primary producer biomass and other macroinvertebrates density in the Colorado River below Glen Canyon Dam in October 2002. Biotic biomass (AFDM g/m²) response variables were Cladophora glomerata (c), detritus (d), Oscillatoria spp.(o), and miscellaneous algae, macrophytes and brophytes (m). Density (#/m²) estimates included Gammarus lacustris (g) megadrile worms (Lumbricidae and Lumbricullidae) (w) , and oligochaetes (Naididae and Tubificidae) (o), chironmonids (ch), simulliids (s), and miscellaneous macroinvertebrates (mm). Only significant response variables are listed (p<0.05). (n=42)

Source	Variable	Coefficient	Probability	Standard Error of Estimate
NZMS x Primary Producers (AFDM/m ²)				
	c	0.004	0.04	0.002
	d	0.036	<0.001	0.004
ANOVA: F _{2,39} =45.5, p<0.001, multiple R ² =0.7				
NZMS x Macroinvertebrate (#/m ²)				
	g	0.52	0.003	0.16
ANOVA: F _{1,40} =10.4, p<0.003, multiple R ² =0.21				
<u>Gammarus</u> x Primary Producers (AFDM/m ²)				
	c	1.61	<0.01	0.28
	d	1.81	0.001	0.34
	o	-0.57	0.04	0.29
ANOVA: F _{3,38} =23.7, p<0.001, multiple R ² =0.65				

Table 5. Epiphytic diatom, New Zealand Mudsail (NZMS) and macroinvertebrate biomass estimates from six cobble bars in the Colorado River below Glen Canyon Dam in October 2002.

Site rkm	Epiphytic Diatom AFDM g/m ² (se±)	NZMS AFDM g/m ² (se±)	Macroinvertebrates AFDM g/m ² (se±)
0.8	2.98 (0.76)	2.91 (1.50)	3.50 (1.17)
3.1	3.56 (0.79)	1.51 (0.50)	0.86 (0.24)
98.6	4.10 (0.69)	0.24 (0.09)	0.10 (0.01)
109.6	2.05 (0.37)	0.34 (0.19)	0.09 (0.04)
202.9	1.43 (0.37)	0.001 (0.001)	0.004 (0.003)
328.8	2.89 (0.34)	0.012 (0.002)	0

DISCUSSION

New Zealand Mudsnaills have colonized in all habitats examined (cobble, silt/sand, macrophytes, and varial zone pools) on the Colorado River through Grand Canyon and have invaded 28% of the tributaries that converge with the main stem. Highest densities were collected from aquatic macrophytes, which may be due to the slower water velocities associated with these habitats. Richards *et al.*, (2001) found that macrophyte habitats on the Snake River, Idaho created refuges for smaller snails and acted as nurseries. Macrophyte habitats are limited on the Colorado River and are restricted to areas with high light penetration and low water velocities. These invasive snails are also commonly found on cobble bars, which is a consistent habitat throughout the main stem. Cobbles and boulders provide attachment surfaces for algae and epiphytic diatoms, a food source for NZMS. Silt/sand habitats are greatly disturbed by high water velocities, fluctuating flows, and migration of sand, which may be why NZMS were rarely observed in this habitat. However, low densities ($< 50/m^2$) were observed in eddies created behind sand ripples where detritus often accumulates on the downs stream side.

Oberlin *et al.*, (1999) reported in a synthesis paper of the 10 major tributaries in Grand Canyon that no snails were collected below the Little Colorado River (rkm 99.1). However, we collected both NZMS and physid snails in the tributaries. This information suggests that the phyto-benthic community is still open to invasions and colonization by exotic taxa (Benenati *et al.*, 1999). It is suspected that recreational activity is the primary cause of dispersal between and within river drainages.

Size class data indicates there were many populations of NZMS. Sexual maturity is reached at 3.0 mm in length, however larger size classes produce more offspring (Richards *et al.*, 2001). All sites had a high percentage of

sexually mature NZMS, however the lack of the large size classes (5.0-5.5 mm) indicate that few have the ability to produce a high density of offspring. This information is important because it shows that these snails can take advantage of micro-habitat conditions to reproduce and that a single management action is not likely to control them.

Although NZMS are established in some tributaries, its ability to move rapidly to new habitats may endanger these pristine habitats. They have invaded tributaries of the Snake River (Bowler 1989, Richards *et al.*, 2001), geothermal rivers of Yellowstone National Park (Hall *et al.*, 2002), Madison River, MT (Cada and Kerans 2002), and the Green River, UT. Tributaries of the Colorado River are important habitats and breeding grounds for the endangered humpback chub; physid snails; a diversity of aquatic macroinvertebrates including: mayflies, caddisflies, dragonflies, and damselflies; native plants; and mammals, which may be endangered by the colonization of the NZMS. Since NZMS prefer habitats with constant temperatures, flows, and high primary productivity, fall flash floods in tributaries may diminish chance of long-term establishment of NZMS. Monitoring of the tributaries for NZMS invasion is important to evaluate the habitat tolerances and potential competition with the native species in these ecosystems.

The effects of NZMS colonization on the Colorado River aquatic ecosystem is unknown. NZMS may directly or indirectly compete with pre-NZMS dominant macroinvertebrates, alter species composition and structure of the primary producers, and disrupt the energy transfer from macroinvertebrates to native and non-native fish. We have already documented a reduction in the epiphytic diatom biomass at Lees Ferry (Table 5) in comparison to pre-NZMS invasion data from 1992 (Benenati, *et al.*, 1999). A study conducted in Darlington Ditch, Madison River, Montana showed that NZMS depressed

periphyton biomass and native macroinvertebrate densities and biomass, especially mayflies, caddisflies, and chironomids (Haynes *et al.*, 1985; Cada and Kerans 2002). In the Colorado River, prior to NZMS invasion, chironomid larvae and Gammarus lacustris provided a critical link between algal and fish trophic levels (Blinn *et al.*, 1992). Competition with these invertebrates by the NZMS may disrupt this critical link.

Although, detecting a loss of taxa, direct impact on native fish or insectivorous birds are doubtful, these invasive snails will contribute to the “sub-lethal but negative impact” similar to what Vinson (2001) describes in his long term analysis of the Green River below Flaming Gorge Dam. Vinson (2001) described how compositional changes can diminish the functionality of an aquatic community. These snails pass through the digestive tracks of trout (Haynes *et al.*, 1985), so fish are unable to incorporate NZMS as a food source. Also, NZMS can survive a six hour passage through the gut of a trout and give birth immediately afterward (Haynes *et al.*, 1985), therefore, fish may act as a transport mechanism for NZMS to unaffected sites. A good term to describe these invasive pests are “trophic dead-end” because they lack an aerial life stage that can contribute to riverine food web as well not being digested by fish. Studies evaluating the competition between the NZMS, Gammarus, chironomids, and primary producers are an important step to understand the effect that NZMS may have on this light driven aquatic ecosystem.

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