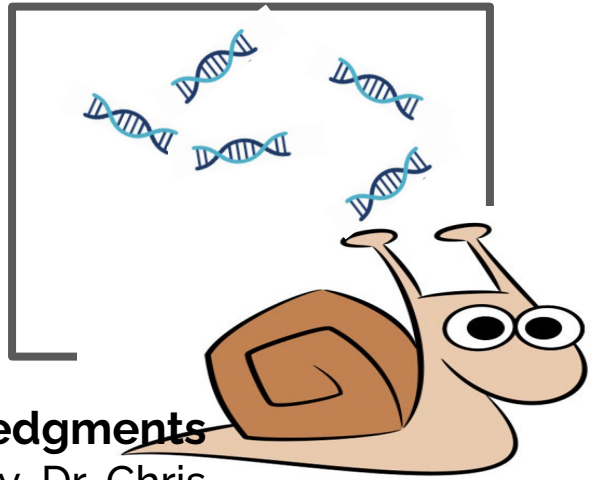
Now You See Them, Now You Don't?

Using eDNA to confirm removal of invasive snails

Dr. Romi Burks
Southwestern University

Coauthors: Cynthia Bashara, Lillian Dolapchiev, Cassidy Reynolds, Esme Rosas Barrientos, Chris Vaughn & Dr. Matthew Barnes



Acknowledgments

The Snail Lab, San Antonio River Authority, Dr. Chris Jerde & Southwestern Biology Department

TEAM AMPLIFIED

Funding:
Southwestern Univ.,
Texas Tech Univ.,
National Science &
Keck Foundations
UM Sam Taylor,
Texas Academy of
Science

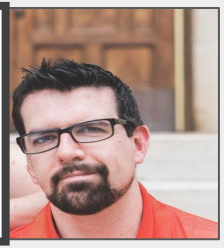
~20 years of snails
10 Years of molecular ecology
20 +undergraduate students
3 molecular collaborators

BURKS' LAB

Dr. Ken Hayes
Bishop Museum, HI
Apple Snails

eDNA Detectives 2022

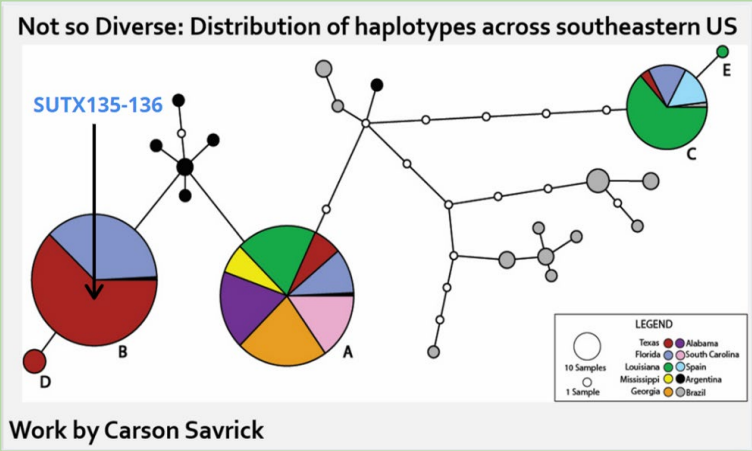
Dr. Matt Barnes '06
Snail Lab Alumni
Texas Tech University
eDNA



Dr. Russ Minton
Gannon University
Mysterysnails

eDNA Detectives 2016-2019

Mystery Team 2018-2020

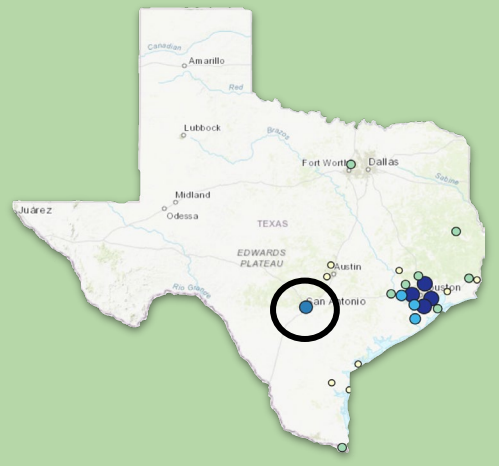


Work by Carson Savrick

Why Apple Snails?

- ***Pomacea maculata***
  - Invasive
- Non-native
  - South America
- Characteristics
  - Gill & lung
  - Warm climate
  - Destructive
  - Fecund

TX map of USGS-NAS records for *P. maculata*. First near Houston, now more snails popping up in TX.



Apple Snails in the News

GOV & POLITICS EDUCATION ARTS & CULTURE BUSINESS & TECH ENVIRONMENT HEALTH WHERE I LIVE BEXAR'S EYE IN MEMORIAM

ENVIRONMENT

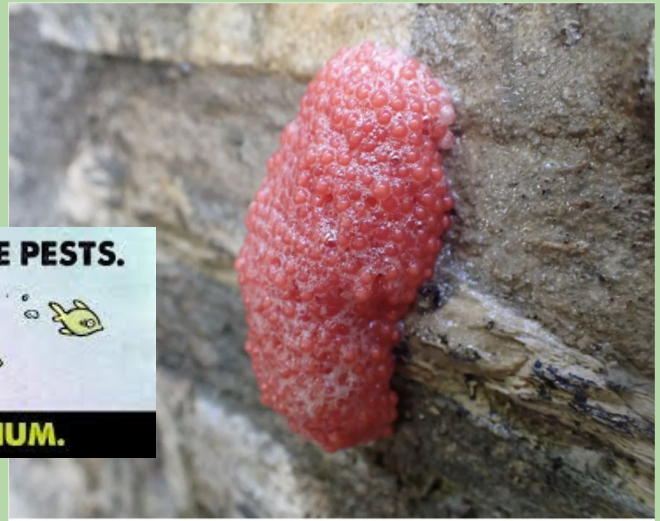
Snails, Tilapia, Scooters Pulled From Drained San Antonio River

BRENDAN GIBBONS | 17 HOURS AGO

LIKE TWEET EMAIL PRINT SHARE COMMENTS MORE

SCOTT BALL / RIVARD REPORT

An invasive nonnative apple snail is held by a San Antonio River Authority employee after it was extracted from the San Antonio River.



Growing Apple Snail Population in San Antonio River Raises Concerns

The invasive species threatens the channeled, low-flow urban stretches of the river, where greenery and shelter for native species are at a premium.

San Antonio Report / Monika Maeckle / Jul 3, 2020

Why care?
Pomacea maculata Reproductive Statistics

2000

Eggs

Average number per clutch

20

Clutches

Per reproductive season in TX (mid-May - October)

70%

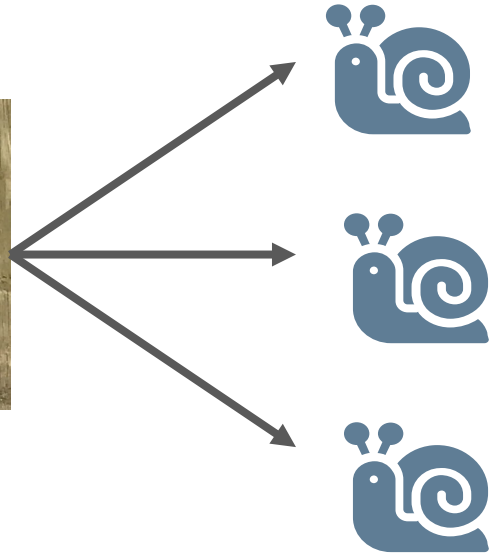
Hatch

Of the clutch yields hatchlings on average

0.0001

Survive

Unknown but consider 99.99% mortality rate



WHAT'S IN A NAME? WHAT'S CHANGED?

2002 — **2012-2015** — **2024**

- "1 of the 100 worst"
- Assumed *P. canaliculata*
- Prohibited in 2002 (TX)
- Spread across SE US started

- Common language
- Regulatory power
- Scientific advancement

The Linnean Society of London — Zoological Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2012, **166**, 723–753. With 14 figures

Comparing apples with apples: clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda)

KENNETH A. HAYES[1*], ROBERT H. COWIE[1], SILVANA C. THIENGO[2] and ELLEN E. STRONG[3]

[1]*Center for Conservation Research and Training, Pacific Biosciences Research Center, University of Hawaii, 3050 Maile Way, Gilmore 408, Honolulu, HI 96822, USA*
[2]*Instituto Oswaldo Cruz/Fiocruz, Av. Brasil 4365, 2104-900 Rio de Janeiro, RJ, Brasil*
[3]*Smithsonian Institution, National Museum of Natural History, P.O. Box 37012, MRC 163, Washington, DC, WA 20013-7012, USA*

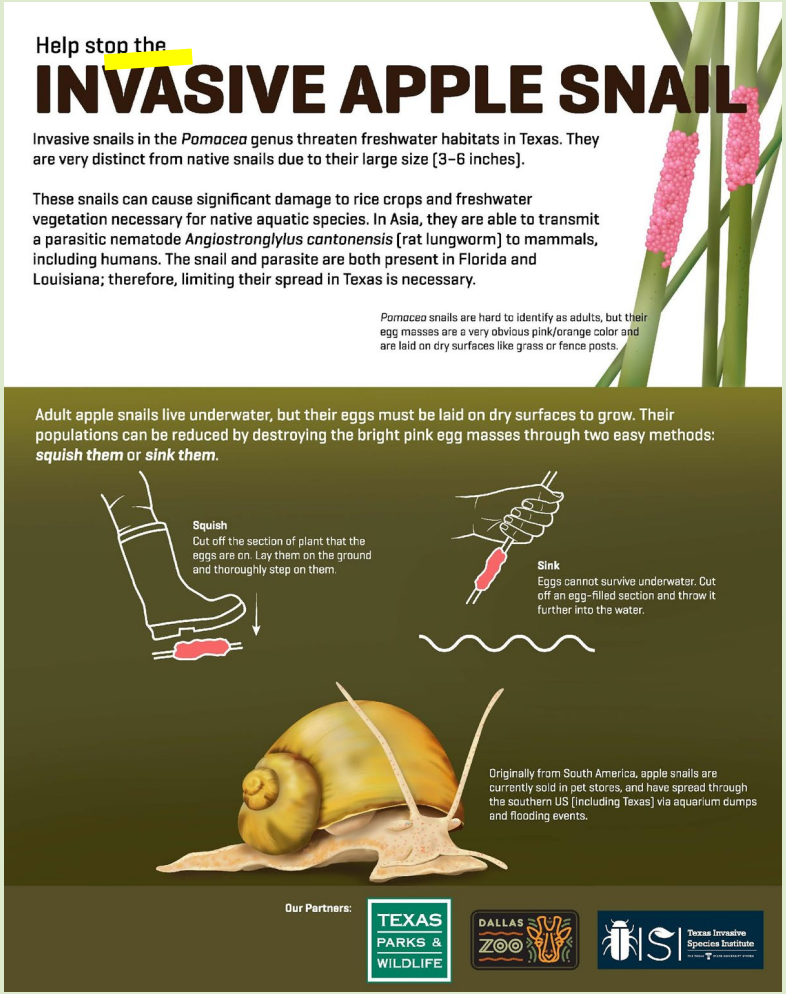
Received 3 May 2012; revised 10 August 2012; accepted for publication 22 August 2012

Synonymizing names:

Pomacea insularum to *Pomacea maculata*

Avoid: channeled, giant, island, golden, GAS

Just say "maculata" apple snail



ENVIRONMENTAL DNA (eDNA)

"Environmental DNA (eDNA) refers to the extracellular, residual DNA shed **from an organism** found in abiotic environments" (Barnes et al. 2014).

QUESTION – Why use environmental DNA when you can "see" apple snails?

SPECIAL REPORT

FUTURE OF CONSERVATION

How Scientists Use Teeny Bits of Leftover DNA to Solve Wildlife Mysteries

Environmental DNA helps biologists track rare, elusive species. It could usher in a revolution for conservation biology

eDNA: A successful technique for identifying cryptic species in a remote location

Thursday, 11 March 2021

Home > Cryptic Tools for Cryptic Species: Using Innovative eDNA Sampling to Find t...

Cryptic Tools for Cryptic Species: Using Innovative eDNA Sampling to Find the Secretive Sharp-tailed Snake

Posted on October 22, 2021

Categories:

BC Parks News, Featured

Environmental DNA

Dedicated to the study and use of environmental DNA for basic and applied sciences

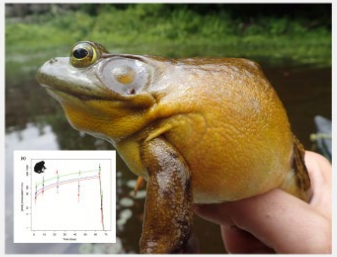
ORIGINAL ARTICLE | Open Access

Detection of a cryptic terrestrial insect using novel eDNA collection techniques

Catriona D. Campbell, Dianne M. Gleeson, Elise M. Furlan, Kate A. Muirhead, Valerie Caron

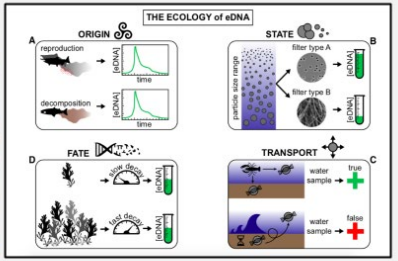
First published: 17 March 2022 | https://doi.org/10.1002/edn3.295 | Citations: 3

TAKE HOME MESSAGE: WE USE eDNA A LOT IN CONSERVATION STUDIES...



Dejean et al. 2012

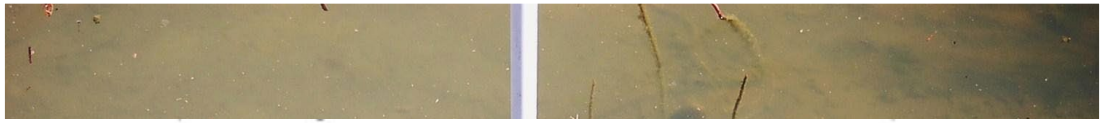
BUT WE DO NOT KNOW ENOUGH ABOUT "ECOLOGY OF eDNA..."



Barnes & Turner 2016

Environmental DNA: Used for cryptic species detection

Not exactly cryptic….or are they? Habitat, season, etc…



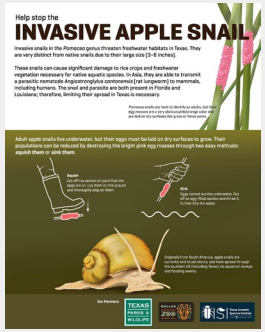
Associated phrases

Crypsis or cryptic	Typically refers to camouflage or behavior(s) related to hiding (e.g., cryptic habitat, cryptic coloration, cryptic mate choice), or unexpected discoveries (e.g., cryptic introduction or cryptic invasion, cryptic genetic variation). 'Morphological crypsis' would describe taxa that are difficult to discriminate using morphology
Cryptic diversity	To allude to more than one cryptic species present in the studied taxon or larger group
Hyper-cryptic	To describe many occurrences of cryptic species in a study group (e.g., Adams et al., 2014)
Cryptic speciation	Probably first cited in the 1950s but not defined (Price, 1958), this indicates evolutionary process(es) leading to the formation of cryptic species (e.g., in gastropods, Fernandes et al., 2021; Sanjuan et al., 1997). It is unclear if this is in reference to one specific, or several different pathways

2002

Using Basic Science to Inform Applied

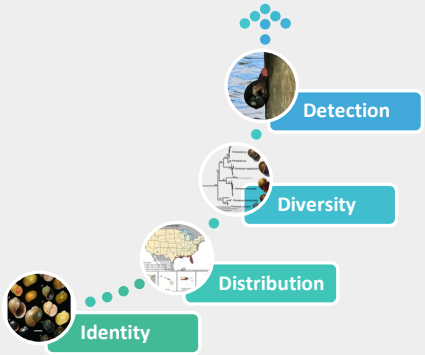
2024



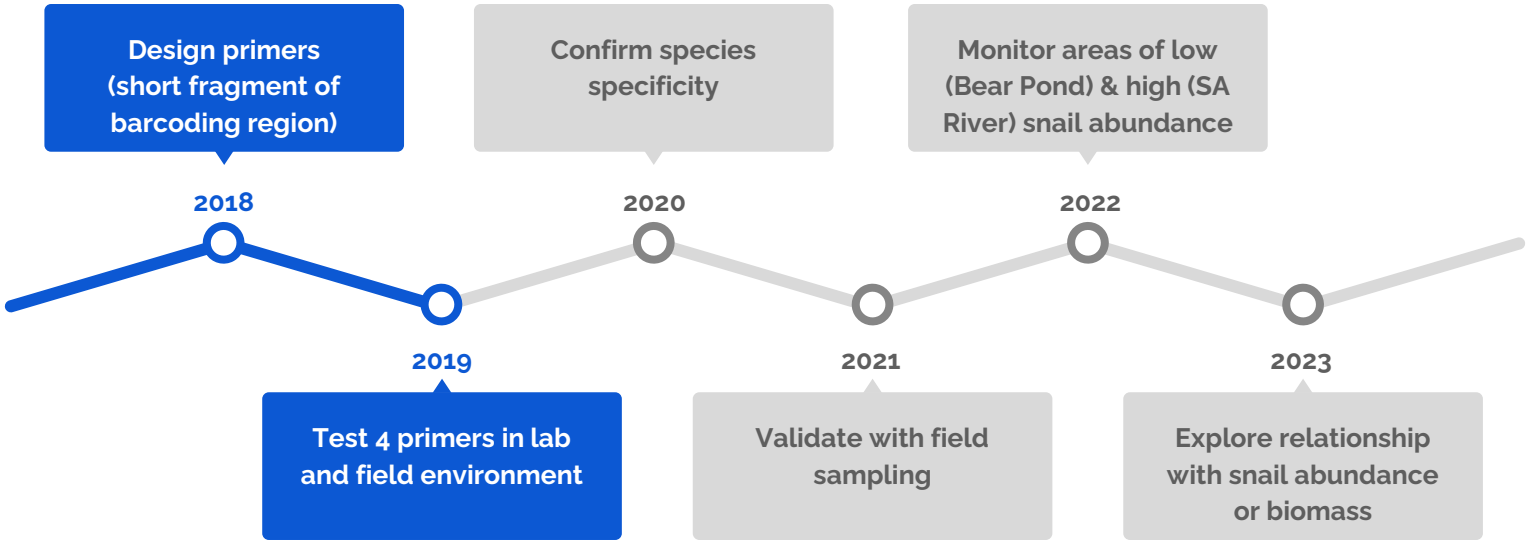
Applied / Conservation

Detection: qPCR and Environmental DNA

Basic / Foundational

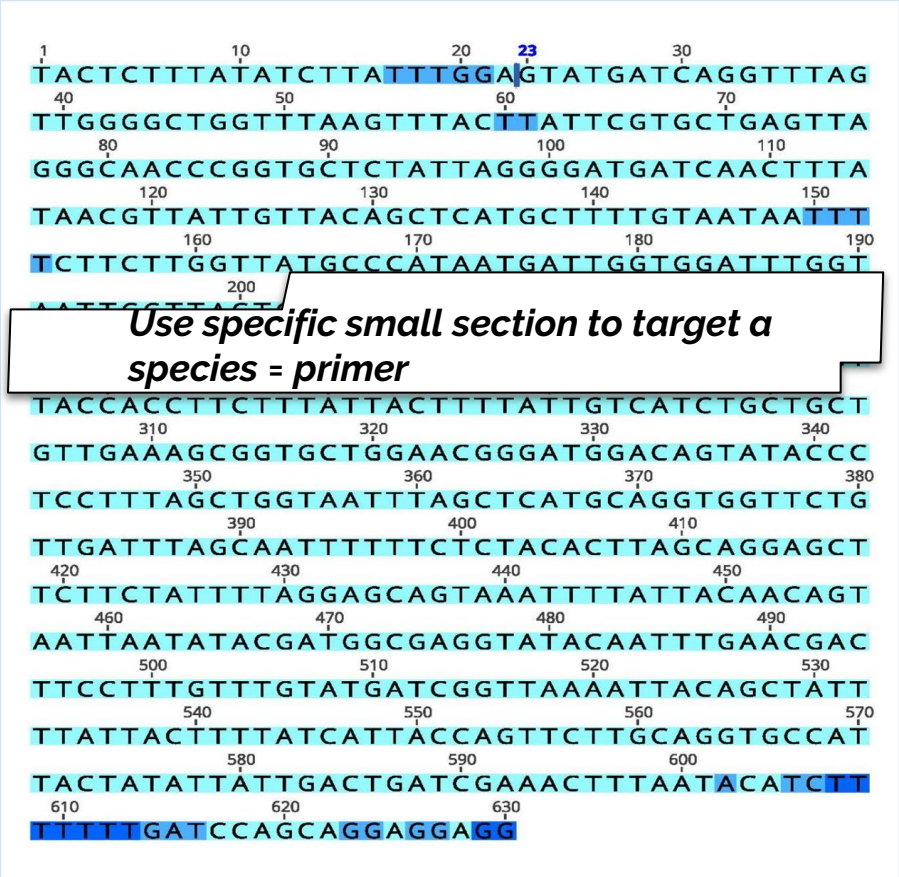


Development of eDNA Detection



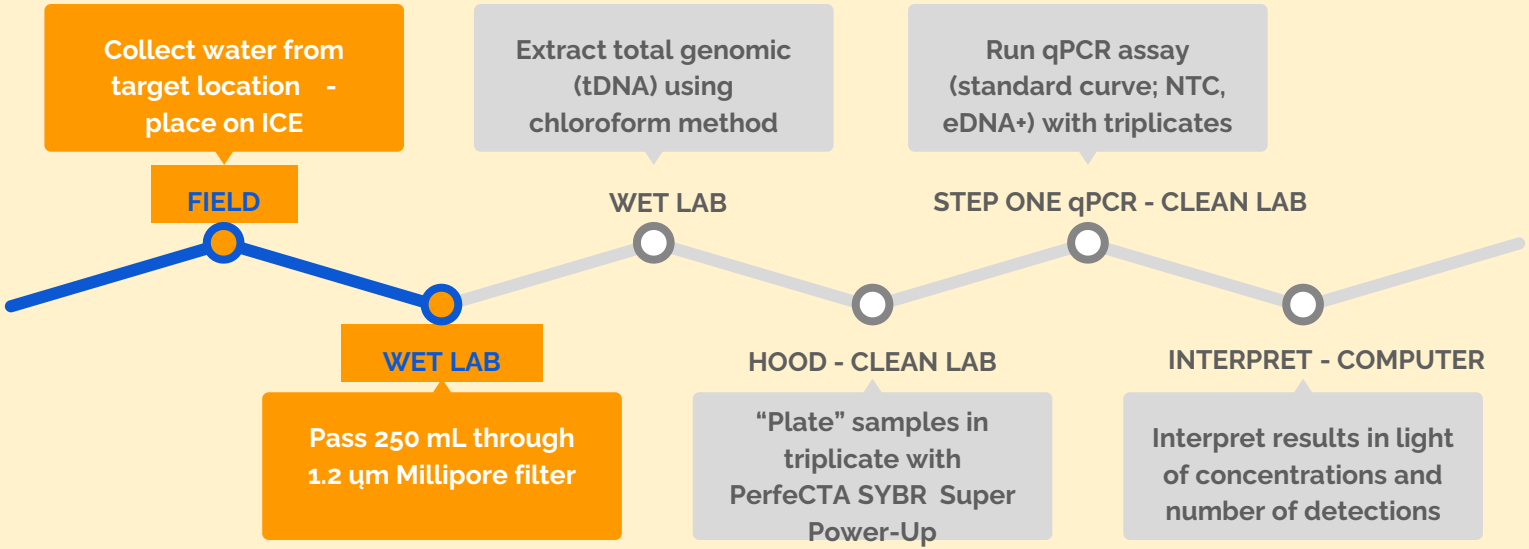
Key points: eDNA done in collaboration with Dr. Matt Barnes; Sequences used based on past collaborations with Dr. Ken Hayes

Although eDNA used to find cryptic species, there's nothing cryptic about its ability to identify to a species-specific level at low abundance.



Working species-specific primers: Banerjee et al. 2022

The Sampling Process



Key points: Verified identities based on past collaborations with Dr. Ken Hayes

Use multiple approaches to address different questions

LABORATORY EXPERIMENTS

FIELD SAMPLING

eDNA WORK

LOCALIZED CASE STUDY

Snail slime in real time: Challenges in predicting the relationship between environmental DNA and apple snail biomass

Romi L. Burks[1], Cassidy Reynolds[1], Esmeralda Rosas[1], Cynthia Bashara[1], Lillian Dolapchiev[1], Christopher L. Jerde[2] and Matthew A. Barnes[3]

[1]*Southwestern University (SU), East University Avenue, Georgetown, TX 78626, USA*
[2]*University of California Santa Barbara (UCSB) Marine Science Institute, Santa Barbara, CA 93106-6150, USA*
[3]*Texas Tech University (TTU), Lubbock, TX 79409, USA*
Corresponding author: Romi L. Burks (burksr@southwestern.edu)

Citation: Burks RL, Reynolds C, Rosas E, Bashara C, Dolapchiev L, Jerde CL, Barnes MA (2024) Snail slime in real time: Challenges in predicting the relationship between environmental DNA and apple snail biomass. *Management of Biological Invasions* 15 (in press)

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Published: 22 July 2024

Thematic editor: Mattias Johansson

Abstract

As environmental DNA (eDNA) becomes a fixture in the invasive species management toolbox, expectations of its utility extend beyond presence/absence to analyses that provide more detailed information about target populations. Studies with fish and other vertebrates have reported moderately reliable, positive relationships between eDNA concentrations and biomass. However, few studies have considered this relationship in invertebrates. To address this gap, we investigated whether increasing biomass of apple snails (*Pomacea maculata*) resulted in a similar predictive relationship with eDNA concentration, and we did so under cold conditions that make apple snails less conspicuous and more difficult to detect with traditional methods. Placing snails in either distilled or stream water, we used a species-specific quantitative PCR assay to measure eDNA concentrations after 24 hr over an apple snail biomass gradient (0, 2, 4, or 6 snails; 143 to 624 g total biomass). Detection success of eDNA derived from apple snails kept in a small volume (i.e., 13 L) of cold water (13 °C) averaged 66% overall. Successful detection in distilled water (75%) exceeded the overall average. Lower detection efficiency occurred in stream water (58%). Despite the cold conditions, we observed snail activity in 90% of our replicates, but net eDNA accumulation failed to reflect patterns commonly observed with vertebrates. Censored regression modeling efforts, which account for a disproportionate number of zeros (i.e., non-detections), identified a significant predictive relationship between snail biomass and eDNA concentration, but only starting at a high amount of biomass (~ 422 g). Future management strategies to monitor apple snails will likely include eDNA, but its utility in ascertaining biomass remains unclear. Considering the ecology of eDNA of invasive invertebrates will help bolster managers' ability to understand the utility and limitations of this valuable tool.

Key words: mollusk, invasive species, *Pomacea*, detection, Tobit

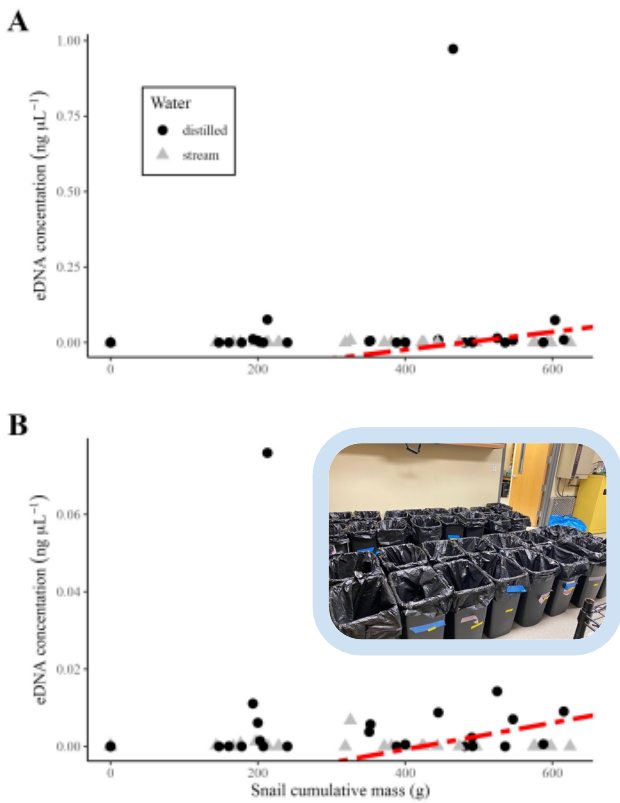


Figure 1. Relationship between net eDNA concentrations (ng μL^{-1}) after 24 hr and cumulative apple snail (*Pomacea maculata*) biomass (g) in a laboratory experiment as a function of snail cumulative biomass (g) in a tank for two water treatment types: distilled (black circles) and stream (gray triangles). One container with four snails amassed a relatively large amount of eDNA compared to all other replicates. The Y-axes display different scales as the top panel (A) includes a statistical outlier while bottom panel (B) omits the outlier. The dashed red line represents the linear relationship suggested by the best-fit Tobit model (M.2), conc = −0.0143 + 0.000296 (mass), for N = 64 (A) and conc = −0.0145 + 0.0000398 (mass), for N = 63 (B), respectively.

Use multiple approaches to address different questions

LABORATORY EXPERIMENTS

FIELD SAMPLING

eDNA WORK

LOCALIZED CASE STUDY

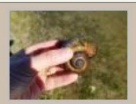
Location: Bear Lake (really a pond!)

USGS Record (1/28/20):

nas.er.usgs.gov/queries/SpecimenViewer.aspx?SpecimenID=1634245

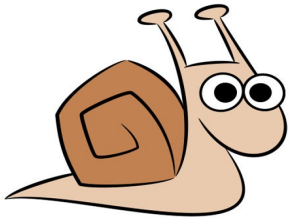
Pomacea maculata
(giant applesnail)
Mollusks-Gastropods
Exotic

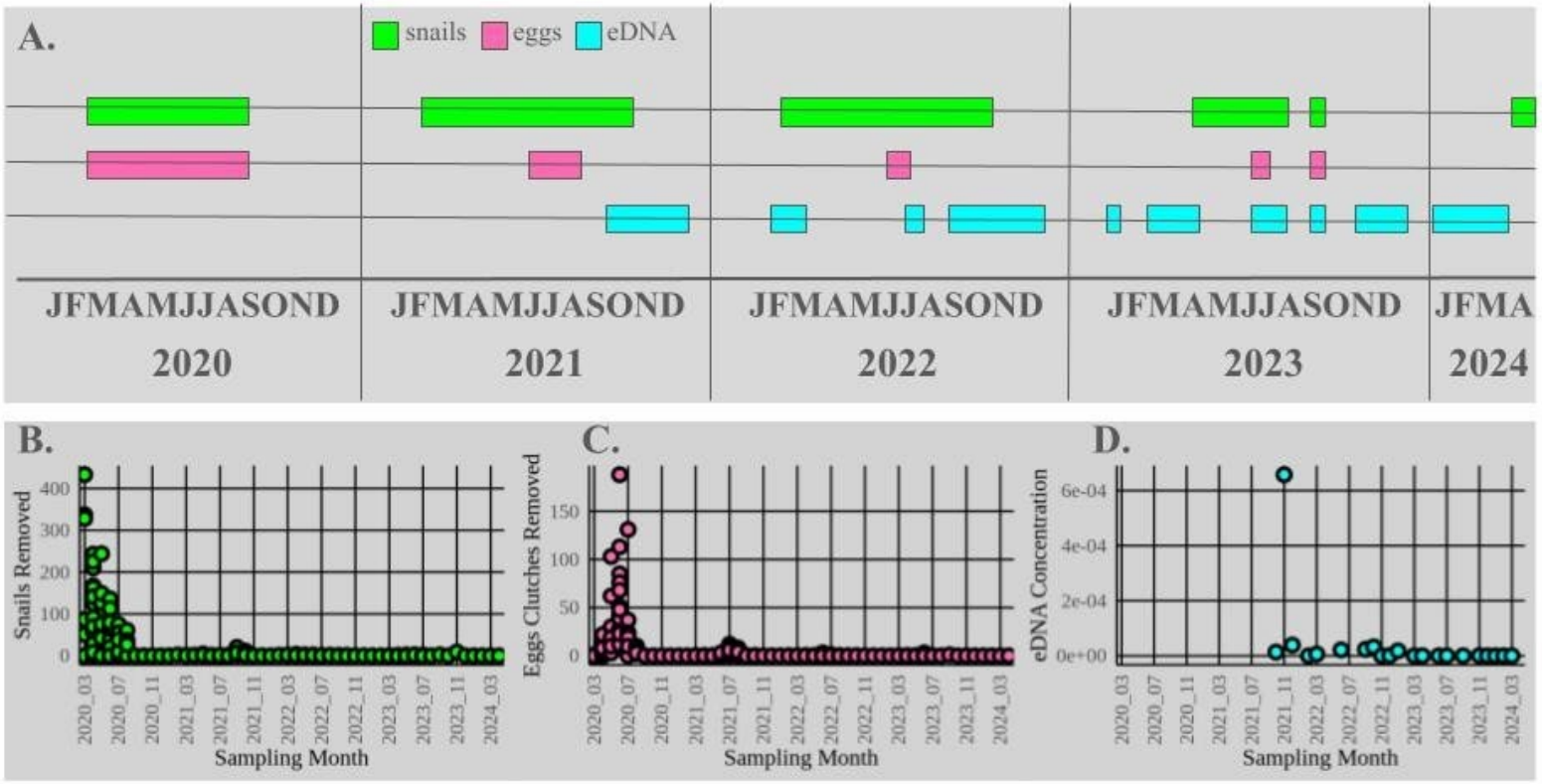
Collection Info
Point Map
Species Profile
Animated Map
Impacts

Collection Day	28
Collection Month	1
Collection Year	2020
Year Accuracy	Actual
Potential Pathway	dispersed | dispersed flood | hitch hiker
Status	unknown
Comments	reported to Monica McGarrity at TPWD by Dave Christie
Record Type	NAS sighting report
Verifier	Monica McGarrity (TPWD)
Freshwater/Marine	Freshwater
Number Collected	3
Photo	 Monica McGarrity  Monica McGarrity

Specimen ID	1634245
Group	Mollusks-Gastropods
Genus	Pomacea
Species	maculata
Common Name	giant applesnail
State	TX
County	Travis
Locality	Bear Lake in the Meridian subdivision, an impoundment of Bear Creek.
Mapping Accuracy	Accurate
HUC8 Name	Austin-Travis Lakes
HUC8 Number	12090205
HUC10 Name	Onion Creek-Colorado River
HUC10 Number	1209020504
HUC12 Name	Bear Creek
HUC12 Number	120902050406
Map	

Citizen Science In action!




A.
snails
eggs
eDNA
JFMAMJJASOND
2020
JFMAMJJASOND
2021
JFMAMJJASOND
2022
JFMAMJJASOND
2023
JFMA
2024
B.
Snails Removed
400
300
200
100
0
2020_03
2020_07
2020_11
2021_03
2021_07
2021_11
2022_03
2022_07
2022_11
2023_03
2023_07
2023_11
2024_03
Sampling Month
C.
Eggs Clutches Removed
150
100
50
0
Sampling Month
D.
eDNA Concentration
6e-04
4e-04
2e-04
0e+00
Sampling Month

NON-DETECTIONS? - AUSTIN, TX

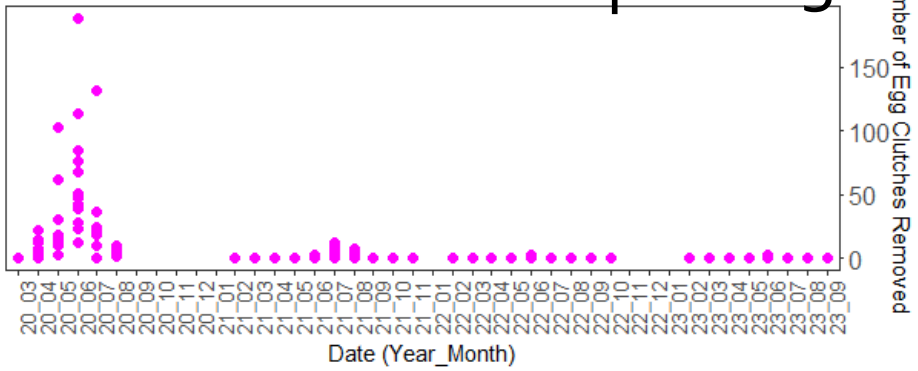
2002 — 2019 — 2024

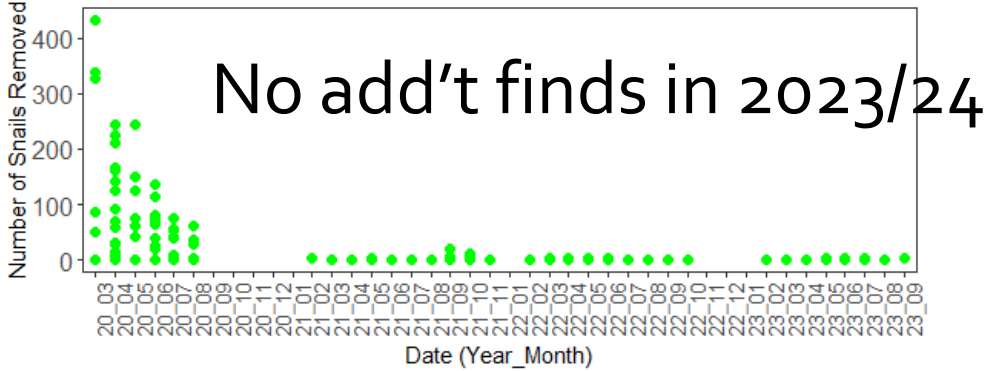
Small pond, Travis County/Austin, TX: Verified species & haplotype; ~5000 snails removed since pandemic

Removals:

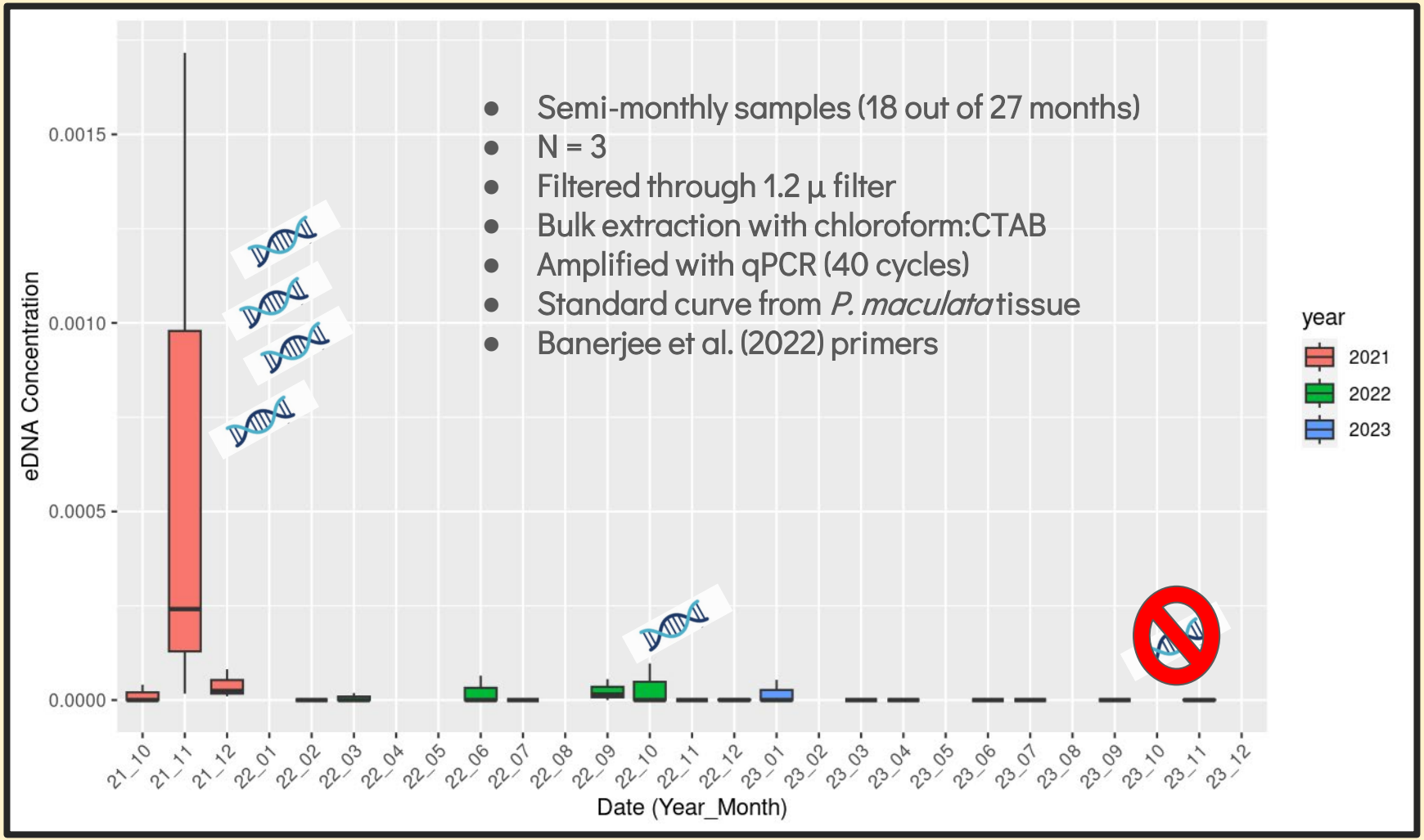
- 2020 Totals: Removed 4779 snails and 1624 egg clutches!
- 2021 Totals: Removed 97 snails and 95 egg clutches.
- 2022 Totals: Removed 53 snails and 8 egg clutches.
- 2023 Totals: Removed 17 snails and 7 egg clutches.
- Grand total: Removed 4946 snails and 1734 egg clutches.

March 2020 - Sept 2023

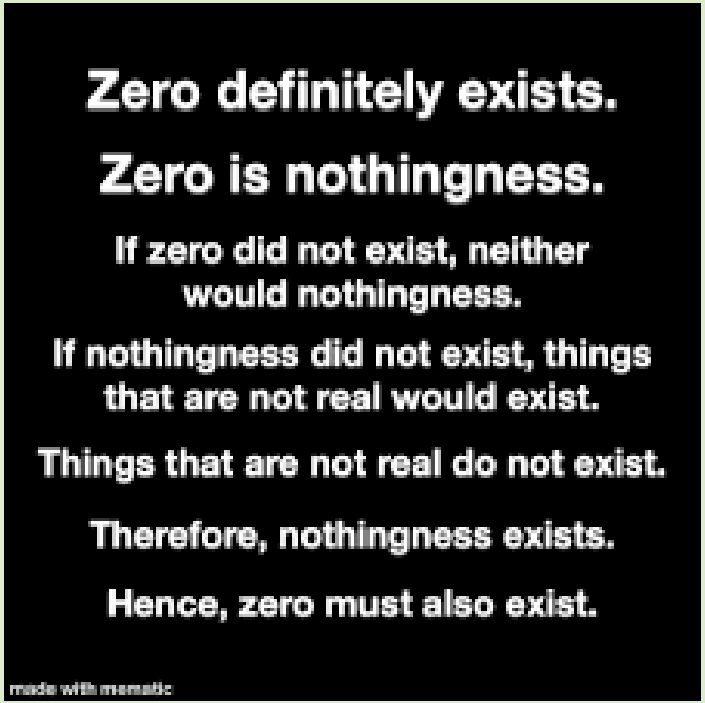




No add't finds in 2023/24


Semi-monthly samples (18 out of 27 months)
N = 3
Filtered through 1.2 µ filter
Bulk extraction with chloroform:CTAB
Amplified with qPCR (40 cycles)
Standard curve from P. maculata tissue
Banerjee et al. (2022) primers
eDNA Concentration
0.0015
0.0010
0.0005
0.0000
21_10
21_11
21_12
22_01
22_02
22_03
22_04
22_05
22_06
22_07
22_08
22_09
22_10
22_11
22_12
23_01
23_02
23_03
23_04
23_05
23_06
23_07
23_08
23_09
23_10
23_11
23_12
Date (Year_Month)
year
2021
2022
2023

How do we know?

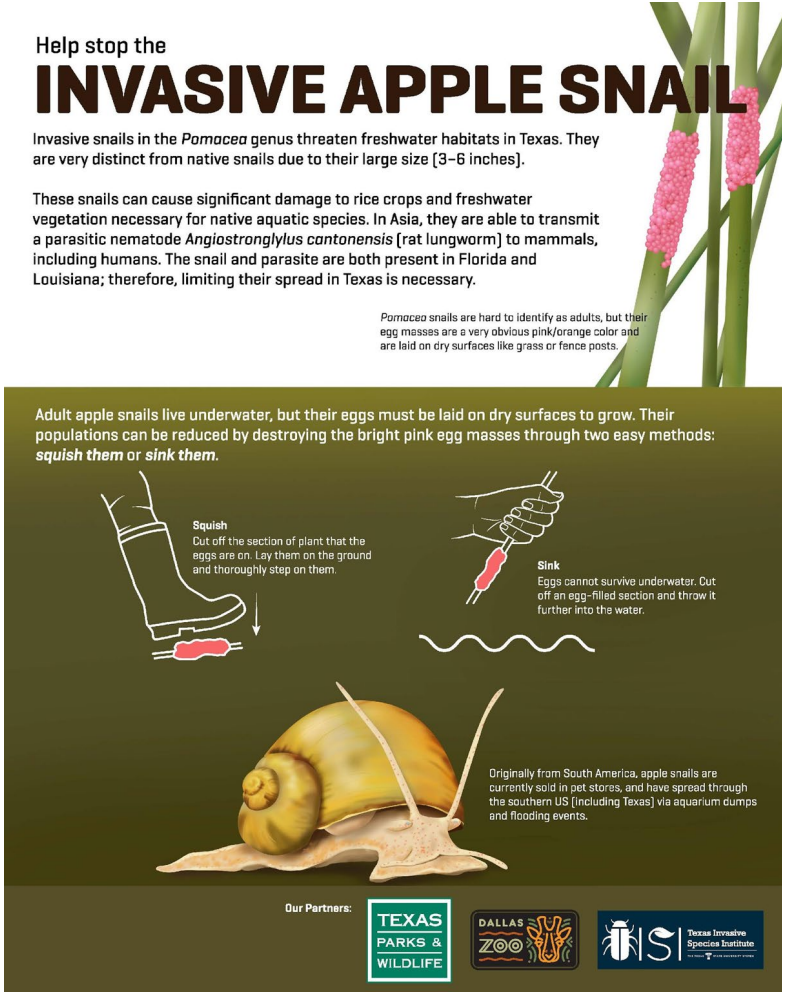


Beyond philosophy,

- We have replicate samples.
- We know these primers work on very low levels of eDNA.
- We used positive & negative controls in our analyses.
- We know the history of the pond.

Take Home Messages

- Power of hand removals for invasive snails.
- Closed systems are rare…apply to rivers?
- Seasonality adds challenges for detection.
- Continue story with minimal monitoring.
- We need to understand what “zero” means.



Take Away: Snails on the move: S-Car-Go(es)….

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